UNIVERSITY OF SASKATCHEWAN

Speciation of Bioavailable Phosphorus in Fluvial Sediments

A Thesis

Submitted to the College of Graduate and Postdoctoral Studies

In Partial Fulfillment of the Requirements For the Degree of Master of Science

Department of Chemical & Biological Engineering

University of Saskatchewan

Saskatoon, SK, Canada

By

Qingxin Zhang

Permission to Use

In presenting this thesis/dissertation in partial fulfillment of the requirements for a postgraduate degree from the University of Saskatchewan, I agree that the libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to:

Head of the Department of Chemical & Biological Engineering

University of Saskatchewan

3B48.5 Engineering Building, 57 Campus Drive

Saskatoon, SK S7N 5A9 Canada

OR

Dean

College of Graduate and Postdoctoral Studies

University of Saskatchewan

116 Thorvaldson Building, 110 Science Place

Saskatoon, SK S7N 5C9 Canada

Abstract

Eutrophication remains a global problem despite more strict regulations. Release of phosphorus (P) from fluvial sediments has shown its huge impacts on downstream water quality, while the P speciation and P release mechanism from fluvial sediments are still not fully understood.

This study applied Dual Culture Diffusion Apparatus (DCDA), chemical sequential extractions (CSE), X-ray Fluorescence (XRF) spectroscopy, and X-ray Absorption Near-edge Structure (XANES) spectroscopy to study the bioavailable P and the transformation associated with two agricultural catchments in Ireland. It is shown that loosely bound P (Loose-P), aluminum bound P (Al-P), and organic matters bound P (OP) are the major contributors to bioavailable P that would promote plankton productions. Other metal ion related P, such as $Ca_3(PO_4)_2$ and $FePO_4\_2H_2O$, are relatively more difficult to be transformed to bioavailable P. The P transformation mechanisms associated with the fluvial sediments from both catchments are showing spatial and temporal differences. The amounts of bio-transferable P in sediments are affected by the pH and the flow rates of the rivers. Besides, the chelation of metal ions oxides with organic matters could inhibit the biodegradations of sediments.

This study provides a conjunctive method for future studies on P speciation and bioavailability associated with fluvial sediments from agricultural catchment. The results could contribute to future sediments source tracing and modeling of the effect of the agricultural activities to the downstream water quality.

Acknowledgement

I would like to express my appreciation to my supervisor, Dr. Yongfeng Hu, for his support and mentoring in my research. I also wish to thank my committee members, Dr. Hui Wang, and Dr. Yen-Han Lin, for providing guidance and advice to my study.

The scope of my research required me to do experiments and sampling in Ireland. I would like to thank Dr. Laurence Gill, Dr. David O'Connell, and Diogo Ferreira for helping me with collecting samples and setting up experimental instruments in Trinity College Dublin, Ireland.

The XANES measurement is performed in the Canadian Light Source. I would like to thank Dr. Qunfeng Xiao and Dr. Mohsen Shakouri for helping me with both measurements and analyses.

This study has been funded by the Natural Sciences and Engineering Research Council of Canada (NSERC).

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Al-P	Aluminum-bound Phosphorus
BB	Ballyboughal
BD	Bicarbonate Dithionite
Ca-P	Calcium-bound Phosphorus
$CaCO_3$-P	$CaCO_3$ associated P
CSE	Chemical Sequential Extraction
DP	Dissolved Phosphorus
DCDA	Dual Culture Diffusion Apparatus
EDTA	Ethylenediaminetetraacetic acid
Fe/Mn-P	Iron/manganese-bound Phosphorus
LCF	Linear Combination Fitting
Loose-P	Loosely bound Phosphorus
OP	Organic Phosphorus
PP	Particulate Phosphorus
P	Phosphorus
SXRMB	Soft X-ray Microcharacterization Beamline
TP	Total Phosphorus
TTA	Tintern Abbey
XANES	X-Ray Absorption Near-Edge Structure
XRF	X-Ray Fluorescence

1 Introduction

1.1 Eutrophication in Agricultural Catchment

Eutrophication refers to a phenomenon where a water body is over enriched with nutrients, due to uncontrolled discharge of municipal wastes and runoff from overuse of fertilization. It is a worldwide environmental problem that may result in harmful algal blooms, oxygen depletion, and fish mortality, all of which have negative impacts on humans in terms of reduced environmental quality, potential health risks and increased management costs (Jennings, et al., 2003). Nitrogen (N) and Phosphorus (P) are the two major nutrients that would contribute to eutrophication, as they are the primary limiting nutrients for plankton growths in water ecosystems (Great Lakes WATER Institute, 2004; Jennings, et al., 2003). Due to the different salinities of coastal water and freshwater, both N and P are limited for plankton productions in coastal ecosystems, while P is the major limiting nutrient for algae in freshwater ecosystems (Ortiz-Reyes & Anex, 2018; Jennings, et al., 2003; Wang & Wang, 2019).

The sources of P causing over-nourished water body include agricultural activities, septic tanks, municipal wastewater, stormwater runoff, industrial release, and fossil fuel combustion (World Resources Institute, 2020). In recent years, the primary source of eutrophication in North America and Europe comes from agricultural activities in fresh water ecosystems, include spreading of animal manures and excessive use of artificial fertilizers (World Resources Institute, 2020; Jennings, et al., 2003). P is not only the limiting nutrient of plankton growths in waters, but also an essential nutrient for plant growths, thus P based fertilizers are required for farming activities (Ghosh, et al., 2015).

P based fertilizers come from limited and non-renewable mineral resources, which, when excessively spread on crops, can be washed off into waters rather than remaining in

soils for agricultural production, resulting in water eutrophications while P shortages in soils (Ulrich, Malley, & Watts, 2016). Therefore, it is critical to manage fertilizer usage in order to achieve a balance towards sustainable use of P reserves, farming activities and eutrophication control. Many studies have been carried out on P cycling and speciation in agricultural soils, while such studies on agricultural catchment stream sediments have not gained as much attentions as soils (Liu, et al., 2017; Kruse, et al., 2015; Ajiboye, 2007; Zhang, et al., 2020). Therefore, to accomplish the goal, it is important to understand the sediment P speciation in agricultural catchment water and sediments.

1.2 Phosphorus in Water

P forms in water body are characterized into dissolved P and Particulate P (PP) (Jennings, et al., 2003). Dissolved P, such as phosphate, is considered a direct bioavailable source for the growth of microorganisms (Jennings, et al., 2003). PPs refer to the P portions in the particles with a size that larger than 0.45μm (Yu, et al., 2012). PP in the forms of fluvial sediments or suspended solids, are not direct sources for algal growth; however, it could contribute to 26% - 75% of total phosphorus (TP) load from agricultural catchments through complex internal P transformation (Jennings, et al., 2003; O'Connell, et al., 2020). PP attached to soil and mineral particles includes both organic and inorganic P forms. Deposits on the riverbed channel are transported as fluvial sediments in streams, and may reintroduce bioavailable P to water systems (Eusterhues, et al., 2014; Hansel, et al., 2005). Evidence has shown that even though the external loading of P has been strictly regulated, the eutrophication remains amplified by the internal P loading, which highlights the importance of studying P transformation mechanisms from internal P sources, such as riverbed sediments and fluvial sediments (O'Connell, et al., 2020).

1.3 Phosphorus in Fluvial Sediments

In regard to fresh water ecosystems, many studies have investigated the internal P transformation in lakes, including both lake water and lakebed sediments (Sondergaard, et al., 2002; Ahlgren, et al., 2005; Orihel, et al., 2017; O'Connell, et al., 2020). Researchers have proven that oxidation-reduction cycle of Fe oxyhydroxide minerals in sediments, as well as interactions of metals (Mn, Al, Ca, Mg, Zn, Cu, Mn) with humic substances, play important roles in phosphate transformation between lakebed sediments and water systems (O'Connell, et al., 2020). Similarly, such interactions may also exist in fluvial suspended sediments of rivers and streams. Compared to lakes, fluvial ecosystems are more dynamic in terms of lower retention time, absence of anoxia and less of buffer ability from surface runoffs (Li, et al., 2014; Jennings, et al., 2003). Therefore, PP might have a greater potential for promoting plankton growths in fluvial systems than in lentic systems. Hence, due to such complexity of the fluvial systems, it is challenging but highly important to study impacts of PP on rivers and/or streams freshwater systems.

1.4 Research Objective and Scope of Research

In order to fill the void of PP's impacts on fluvial systems, it is important to study the chemical composition and transformation of P at a molecular level, particularly in terms of its bioavailability, in fluvial sediments from agricultural catchments.

The goal of this study is to investigate the P transformation of the fluvial suspended sediments from agricultural catchments, and expand our understanding in the mechanisms of P speciation transformation in fluvial system.

This thesis is a part of an international Water Joint Programming Initiative Project, which with three countries, Canada, Ireland and Sweden, coordinated together in the area of fundamental analysis, sampling, and modeling, to study the mechanisms of P transformations, trace P sources, and help guiding the farming activities. The scope of the thesis includes

using different analytical methods in conjunction to study P speciation, transformation and bioavailability associated with fluvial suspended sediments in rivers within the geologically contrasting agricultural catchments. The Dual Culture Diffusion Apparatus (DCDA), which is a technical setup that can determine the bioavailable amount of P in suspended sediments, was applied to monitor the bioavailable P release of the suspended sediment samples from geologically contrasting agricultural catchments. The pre- and post- DCDA samples were analyzed using chemical sequential extractions (CSE), X-Ray Fluorescence (XRF) spectroscopy and P-K edge X-Ray Absorption Near-Edge Structure (XANES) spectroscopy in conjunction and comparison to examine the P transformations. CSE is widely used in studying P fractions in soils and sediments, which is relatively simple and feasible. However, with the use of different chemicals in extraction, it could be chemically destructive for some samples, and the results lack composition details (Gu, et al., 2019; Liu, et al., 2017). XRF spectroscopy is able to provide elemental information of samples in relation to relative quantity and correlations. Compared to CSE and XRF, XANES is chemical sensitive, and allows the examination of P speciation at a molecular level. Through processing the XANES spectra, it is able to distinguish and quantify P compositions by fingerprinting analysis and Linear Combination Fitting (LCF). By combining such methods, our goal is to provide an in-depth understanding of P speciation. To the best of our knowledge, no other studies that have attempted to couple all the techniques mentioned to study the P speciation, transformation and bioavailability associated with suspended fluvial sediments from geologically contrasting agricultural catchments.

Hypotheses of the study include that the P in suspended sediments of fluvial systems from agricultural catchment could become bioavailable and promote algal growth; samples from different catchments would show different transformational behaviors depending on the types of catchments and the chemical forms of P and mineral compositions; and the

mechanism of P transformation is sensitive to the interaction between metal oxides and organic matters.

This study focuses on the methodology and analysis of results in terms of P speciation. The results could contribute to future catchment scale PP, source tracing, and predictive modeling, as well as informing further best management practices for farmers to improve downstream water quality.

This study is not assessing eutrophication states of the rivers from the catchment or the fertility levels of the soils. This study does not discuss eutrophication remediation.

1.5 Thesis Outline

The introduction section to this thesis provides the background of eutrophication from agricultural catchments, the importance of the study, the objective, as well as the scope of the study. The Literature Review section summarizes the approaches that could be applied to measure the bioavailable P in sediments, and the techniques that could be used in analyzing the fractions of P in samples. The pros and cons of different approaches are compared, and the reasons of the selections are discussed. The Material and Methods section describes the detailed procedures of the methods used in this study. In the Results and Discussions section, the results of bioavailable P in the sediment, chemical information of the samples, and the understandings of the results are provided. At last, conclusions of the study are highlighted, and recommendations for future studies are outlined.

2 Literature Review

2.1 Bioavailable P in fluvial sediments

In order to understand the mechanisms of P speciation dynamics in suspended fluvial sediments, the first step is to estimate the bioavailable potion of TP in fluvial sediments. Mayer (1985) summarized the common forms of P in particulate sources (such as soils and sediments), and the chemical reagents used to extract those P forms. The review report included some studies that estimate bioavailable P by correlating the results of chemical extractions with the results of a bioassay technique, which was first developed by US-EPA (1971) that directly measures the amount of P consumed by algal species. The conclusion from Mayer's study (1985) is that the bioassay technique is time consuming and costly, so it was concluded that citrate-dithionite-bicarbonate and NaOH-extractable P provide a good estimation of bioavailable P in particulate sources. However, in a later report, Mayer (1991) suggested that the bioavailable P results generated from citrate-dithionite-bicarbonate extractions have a poor reproducibility of 13.5% variation. Nevertheless, due to the time and cost efficiency of chemical extractions, citrate-dithionite-bicarbonate is still used in many recent studies to estimate bioavailable P on particulate sources (Wang, et al., 2012; Soliman, El Zokm, & Okbah, 2017; Zhang, et al., 2020).

Miller et al. (1978) introduced algal bioassays to assess the algal bioavailable P of sediments in the soluble phase. In this method, P-starved algae and wet sediment samples are introduced to sterilized flasks that filled with P-free algal growth medium. The bioassays last for 14 days under 24±2 °C and a 24-hr fluorescent lighting of 4300 lm±10%. The percentage of bioavailable P in particulate sediments is determined by back calculating the increased algal cell counts. This method is convenient to estimate the percentage of bioavailable P in fluvial sediments, and has been used by Ellison and Brett (2006) in conjugation with acid persulfate digested TP to estimate the concentration of bioavailable P. However, it is possible

that 14 days of incubation might not be suitable for some samples, which might include sediments with a slower P transformation rate due to the different types of soil from upstream. Besides, it is unable to track the transformation rate during the experiment, since only the TP concentration of sediments is measured pre- and post-commencement of the experiment.

To overcome the disadvantages of algal bioassays, DePinto et al. (1981) designed the Dual Culture Diffusion Apparatus (DCDA) to test the algal bioavailable P in sediments (Figure 2.1).

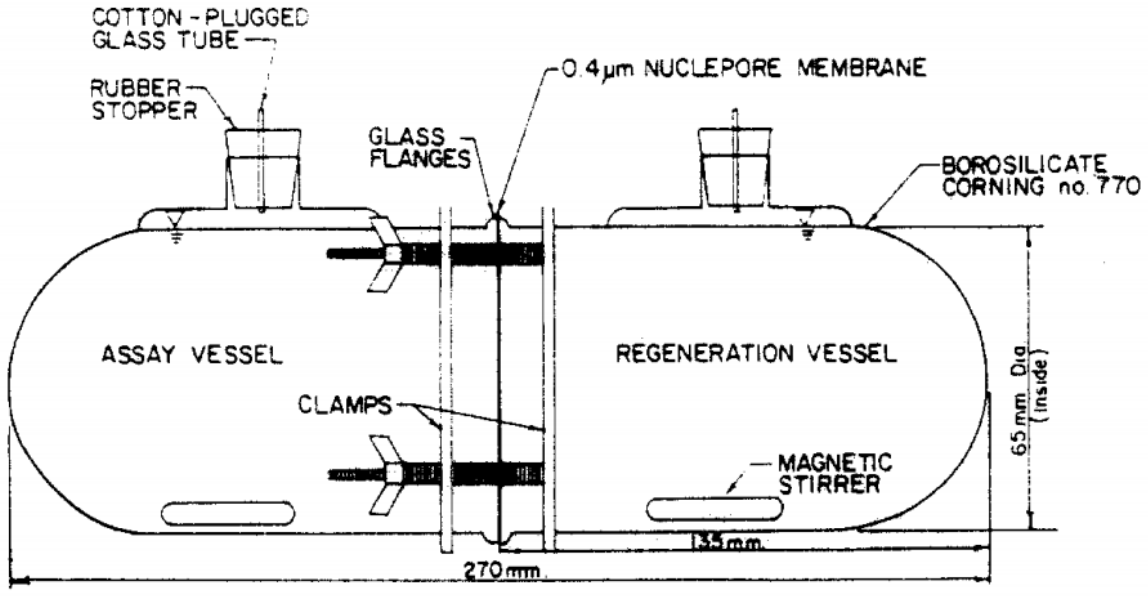

Figure 2.1 A schematic diagram of Dual Culture Diffusion Apparatus (DCDA). Figure is reprinted with permission (DePinto, Young, & Martin, 1981)

The apparatus consists of two borosilicate chambers: one is kept dark with sediments samples, while the other is incubating P-starved algae. Both chambers contain P-free algal growth medium. The whole apparatus is agitated with magnetic stirrers and exposed to constant fluorescent light. The difference of DCDA with algal bioassays is that the algae in the incubation chamber are changed every 3 days. The old algae are harvested and tested for TP, while new P-starved algae are added each time. In this way, it allows tracking the change of P transformation rate while all bioavailable P is consumed by healthy algae at the end of the experiment. Besides, the fluvial sediments can be recollected after the experiment, which

allows comparing the P fractions of sediments from both pre- and post-DCDA experiment. DCDA measures bioavailable P directly, does not involve chemical treatment, and allows continuous experimenting. Therefore, it is a suitable technique for this study.

2.2 Sediments P analyzing methods

Acksel (2019) summarized and reviewed the methods that are currently used in studying P characterization, which are chemical sequential fration (CSE), ^{31}P nuclear magnetic resonance (^{31}P NMR) and P K-edge X-ray absorption near-dege structure (P K-edge XANES) spectroscopy. These methods have their pros and cons, and are utilized in conjuction recently (Acksel, et al., 2019).

2.2.1 Chemical Sequential Extractions (CSE)

Chemical sequential extraction is the most commonly applied method to separate different forms of P in sediments, and the methods have been modified over decades to fit for different samples and studies (Acksel, et al., 2019). Huang's study (2015) summarized the major procedures of CSE developed by different researchers. In general, P in sediments can be sub-categorized into six groups with respect to their sorptivities with different materials in chemical extractions: labile P, iron-bound P (Fe-P), aluminum-bound P (Al-P), organic-P (OP), calcium-bound P (Ca-P), and residue P (Huang, 2015). A common CSE scheme proposed by Psenner et al. (1984) used water to extract labile P, $NaHCO_3$ and $Na_2S_2O_4$ (BD) to extract Fe and Mg related P or reductant soluble P, NaOH to extract Al-P and humic related P, HCl to extract apatite and Ca related P, and 85°C of NaOH to extract residue-P. One of the shortcommings of Psenner's scheme is the underestimations of P amount due to phosphate resoption by carbonates in calcium-rich sediments (Pettersson, Bostrom, & Jacobsen, 1988). To overcome this shortcoming, following researchers have improved the scheme, by increasing the volume-to-solid ratio during alkaline extraction to achieve a better

recovery rate of P in calcium-rich sediments (Hupfer, et al., 2009). Besides, other studies have improved Psenner's methods by adding a digestion step after extraction with NaOH to separate Al-P and OP (Rydin, 2000), and using ignition at 550 °C and boiling samples in 1 M HCl as the last step to maximize the extraction of residual P (Lukkari, Leivuori, & Hartikainen, 2007). However, the term "residue-P" is somehow misleading as "residue" means "something that remains after most of a substance has gone or been removed" (Residue, n.d.). Also, according to Ajiboye (2007), concentrated HCl is able to extract some complex Ca-P and Fe-P. Therefore, the term of "residue-P" is not accurate, and should be changed to other-P.

The deficiencies of CSE include inaccurate results due to degradations from reagents to the samples, and the lack of detailed chemical information (Acksel, et al., 2019; Gu, et al., 2019). However, despite of the shortcomings, CSE provides reliable results of TP for samples, especially for alkaline samples (Acksel, et al., 2019). Also, as it is still widely used by farmers, using CSE in this thesis will provide a basic understanding of the P speciation in sediments, and help comparing academic results with practical results used by farmers, so that it is possible to generate understandable farming guidance. The CSE scheme that is applied in this study is based on Psenner (1984)'s scheme with modifications from Rydin (2000), Lukkari et al. (2007), and Hupter (2009).

2.2.2 ^{31}P nuclear magnetic resonance (^{31}P NMR) Spectroscopy

The basics of nuclear magnetic resonance (NMR) have been well documented, i.e., as explained by Nelson (2003): the nucleus with odd numbers of nucleons has intrinsic spins, and could align either toward or against the magnetic field when applied with strong magnetic field. The direction of the alignment depends on the energy level of the nuclei, i.e. nuclei with higher energy level point against the magnetic field, while the nuclei with lower energy level point toward the magnetic field. The nuclei with lower energy level could be

irradiated to higher energy level if a certain electromagnetic frequency is applied to the nuclei. On the contrary, certain amount of energy would be emitted if the nuclei with higher energy level drop to lower energy level. The resonant frequency of a nucleus is recorded relatively to the frequency of a reference (e.g. 85% phosphoric acid) as chemical shift, which has a unit of ppm (Wilson, 1987).

The chemical shift of the reference is set to be 0 ppm (Hoffman, 2003). The chemical shift of each peak in the NMR spectrum is used to fingerprint the compounds in the samples. An example of ^{31}P NMR spectrum is shown in Figure 2.2, and a reference list of chemical shifts for P-related organic compounds is shown in Table 2.1.

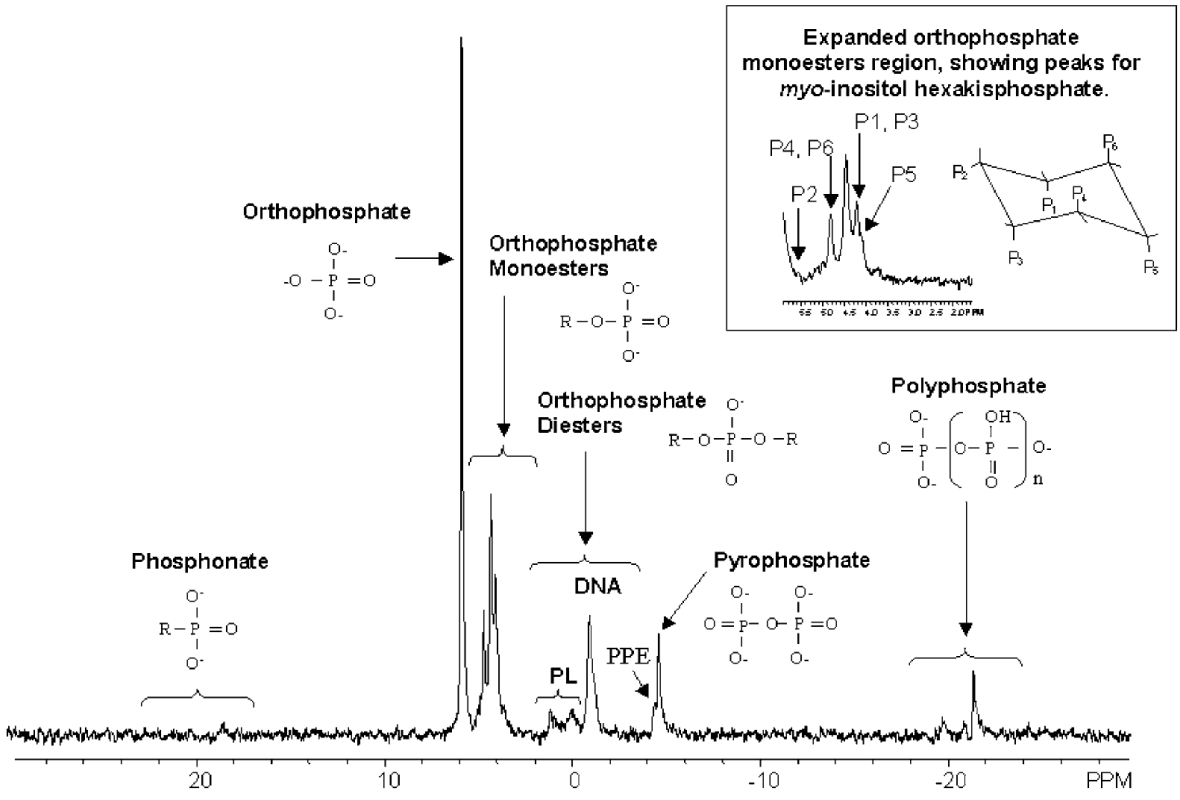

Figure 2.2 An example of ^{31}P NMR spectrum. Figure is reprinted with permission (Cade-Menun, 2006)

Table 2.1 Chemical Shift references for alkaline extracts (pH 12) of organic P compounds. Table is reprinted with permission (Cade-Menun, 2006)

Compound	Chemical shift (ppm)
Phosphonates	20
Aminoethyl phosphonates	20
Phosphonolipids	18
Aromatic phosphonic acid esters	12 to 14
Aromatic diesters	7.4
Orthophosphate	5.7 to 6.1
Orthophosphate monoesters	6-3
Myo-inositol hexakiphosphate (phytic acid)	5.85,4.92,4.55,4.43
Glucose-6-phosphate	5.4
Mononucleotides	4.78 to 4.32
B-glycerophophate	4.85
Ethanolamine phophate	4.71
Scyllo-inositol hexakisphosphate	4.14
Choline phosphate	4.05
Glucose-1-Phosphate	3.2
Orthophosphate diesters	2.5 to -1.0
Teichoic acids	2.5 to 1.2
Phosphatidyl ethanolamine	1.75
Phosphatidyl serine	1.57
Phosphatidyl choline	0.78
RNA	0.54
DNA	0
DNA	-0.37
Polyphosphate terminal P group	-4
Pyrophosphate	-4 to-5
ATP or ADP α-phosphate	-10
Polyphosphates	-19 to-21
ATP β-Phosphate	-19.68

^{31}P NMR spectroscopy is an established technique for identification of organic species in samples (Acksel, et al., 2019). However, as NMR generates structural information by probing the nuclei, it lacks valence information of samples. Besides, even though solution NMR produces better resolutions of spectroscopy, it requires chemical treatment before testing, which might affect the degradation and recovery rate of P in samples (Acksel, et al., 2019). Due to the complexity of ^{31}P NMR spectroscopy, it is not applied in this study. However, in the scope of the project, it is also applied to help understanding P speciation in fluvial sediments at a molecular level.

2.2.3 X-ray Absorption Near-edge Structure (XANES) Spectroscopy

XANES spectroscopy is a synchrotron radiation based technique. Synchrotron is a large-scale facility that electrons are accelerated to nearly speed of light, then injected and stored in the storage ring, where the high-energy electron beam is bent by magnetic field, and emitting radiations at the tangent of the electron path (Usask, 2014). Those radiations, delivered through different beamlines, are designed for use with different techniques (Figure 2.3).

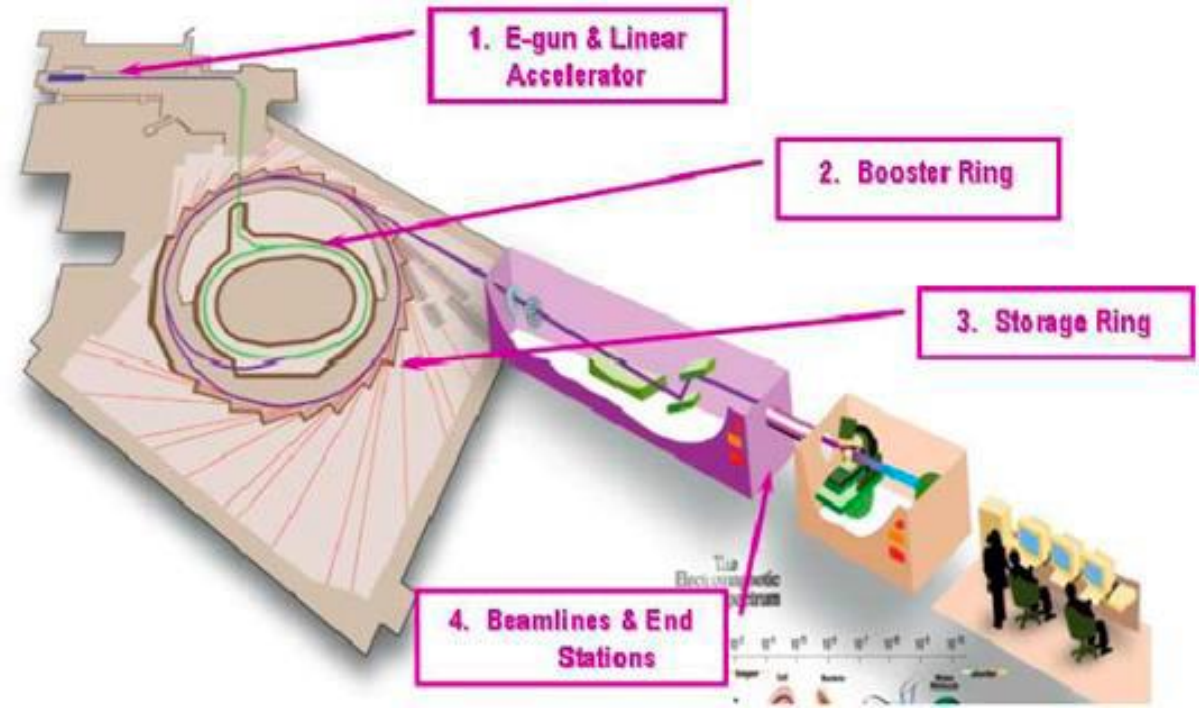

Figure 2.3 Synchrotron radiation facility schematic of Canadian Light Source. Figure is reprinted with permission (Huang, 2015)

XANES uses X-ray to excite electrons on the sample and measures the process of absorbed X-rays. Instead of measuring the energy of fluorescent light only, it also measure the energy of photoelectron and auger electron due to the movement of the electron from higher energy shell to lower energy orbital shell (Figure 2.4).

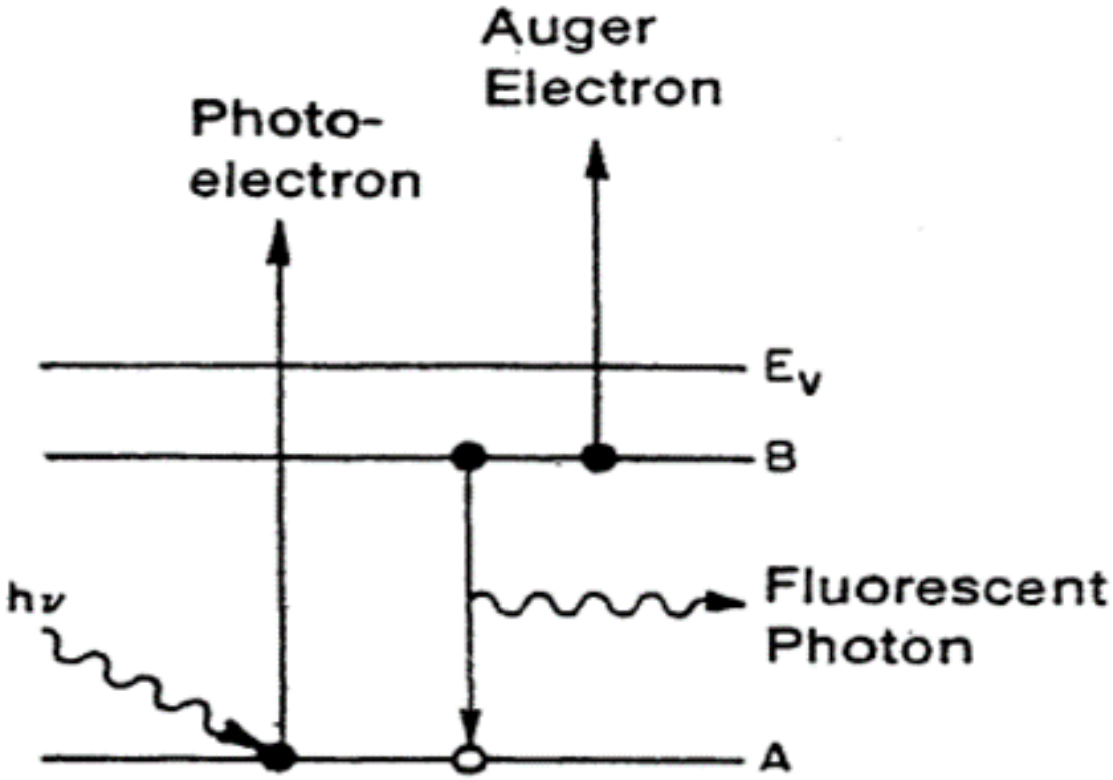

Figure 2.4 Electronic and energy transition for electron movement from high-energy orbital shell to low energy orbital shell. Figure is reprinted with permission (Huang, 2015)

By recording the signal intensity over a range of energy, an X-ray absorption spectroscopy spectrum is generated, which includes pre-edge structure, X-ray absorption near-edge structure (i.e. XANES), and extended X-ray absorption fine structure (i.e. EXAFS) (Figure 2.5). The difference between XANES and EXAFS is that XANES provides the information about the valence state and bonding, while EXAFS gives interatomic information. The spectrum is named as core electron is excited from certain core orbitals (K, L, or M) to the unoccupied state or continuum, for example P-K edge (e.g. for excitation of P 1s electron), Ca-K edge, and Fe-K edge.

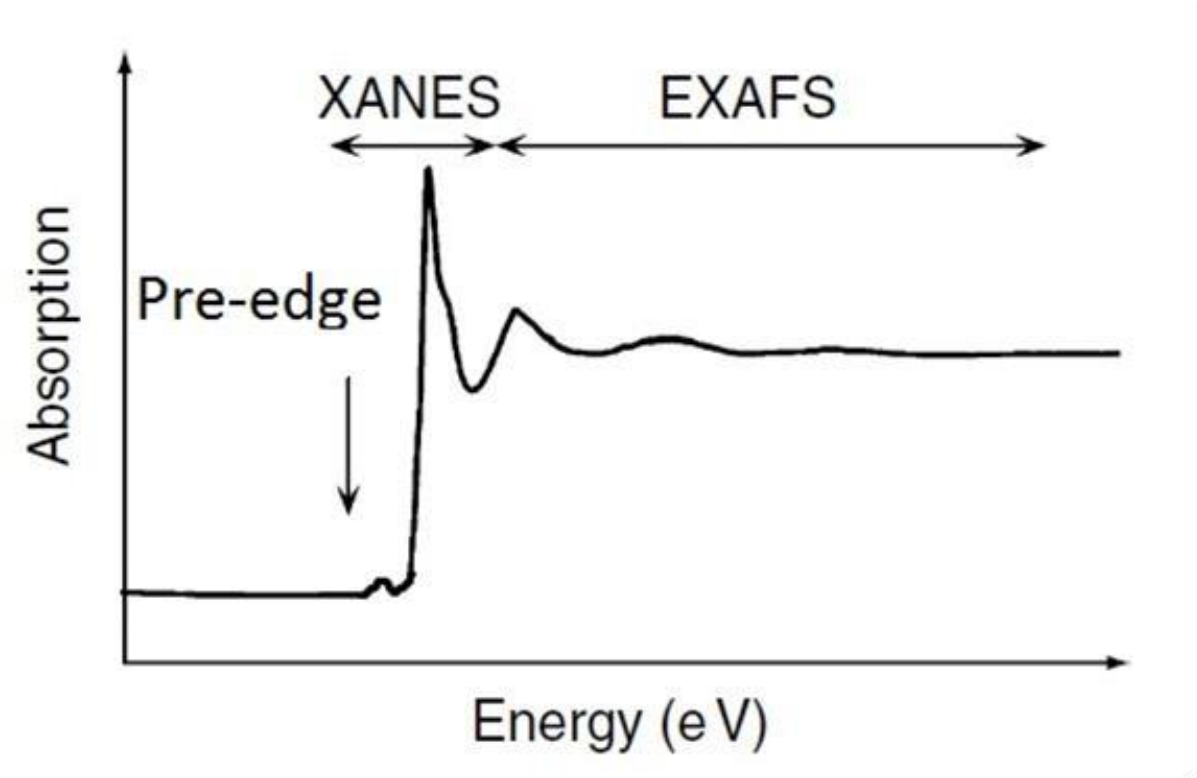

Figure 2.5 An X-ray absorption spectroscopy spectrum. Figure is reprinted with permission (Huang, 2015)

The utilization of XANES spectra in studying P speciation in sediments gives a better understanding of the inorganic form of P. Without adding chemicals in analysis, it could largely minimize the possible chemical interferences associated with the Chemical Sequential Extraction of P, and provide more detailed information of compounds associated with different metals. Also, by comparing to the unique XANES spectra of inorganic P references, it is possible to do fingerprinting analysis that identifies the metal-related compound in samples. It can also be quantified by conducting Linear Combination Fitting (LCF).

2.3 X-ray Fluorescence (XRF) Spectroscopy

XRF is a technique that utilizes X-ray to excite an atom of liquid or solid samples, and measures the fluorescent energy emitted by the electron that moves from higher energy orbital shell to lower energy orbital shell due to the excitation (Verma, 2007). Figure 2.6 is a schematic diagram that shows the XRF analysis using different source types.

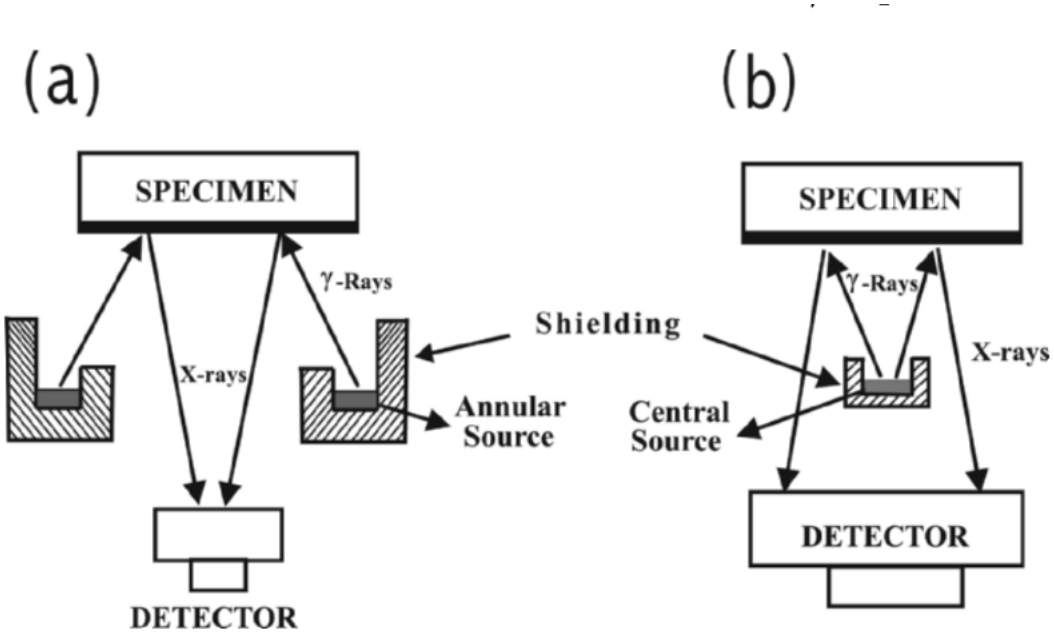

Figure 2.6 A schematic diagram of X-ray fluorescence (XRF) analysis using (a) annular source and (b) central source. Figure is reprinted with permission (Verma, 2007)

XRF is a non-destructive method that allows semi-quantitative analysis for multiple elements with a small amount of samples (Verma, 2007). XRF determines the elemental composition of materials, but is not chemical sensitive, and lack of compound details. In this study, XRF is applied to determine the elemental information of samples, which helps to interpret XANES spectroscopy.

2.4 Summary of Literature Review

CSE is a widely used technique for measuring P in soils and sediments, and has been improved over decades (Psenner, et al., 1984; Rydin, 2000; Pettersson, et al., 1988; Lukkari, et al., 2007; Hupfer, et al., 2009). Despite of the under- and overestimation due to chemical treatment, it is still a useful tool in terms of investigating the operationally defined P andtotal P in sediment samples (Gu, et al., 2019; Acksel, et al., 2019). To complement the shortcomings of CSE, ^{31}P NMR and P K-edge XANES are more advanced techniques with less or no chemical dustructions to study sediment samples with each own strength in

identifying certain types of species (i.e. organic or inorganic). Most of the conjuctive studies of the techniques have been applied in soils, organic amendment, and marine or lake sediments (Ajiboye, 2007; Gu, et al., 2019; Huang, 2015; Defforey & Paytan, 2018). There has been fewer studies in the area of suspended fluvial sediments investigation, especially in studying the P speciation at a molecular level. By comparing the P species and fractions for both pre- and post- consumption of the bioavailable P by applying DCDA, the P transformation under chemical weathering could be simulated, helping to understand the P transformation mechanisms in rivers and streams.

The main objective of this study is to investigate the P speciation dynamics of the fluvial sediments from agricultural catchments by comparing the P species and fractions for pre- and post- DCDA experiment.

The sub-objectives for this study are to investigate the consumption of bioavailable P from fluvial suspended sediments by applying DCDA experiments, and to identify the fractions of P in fluvial sediments by using CSE and XANES in conjunction.

3 Materials and Methods

3.1 Site Selection

Samples are collected from two agricultural catchments of contrasting geology in Ireland, Ballyboughal (BB) and Tintern Abbey (TTA). Locations are shown in Figure 3.1.

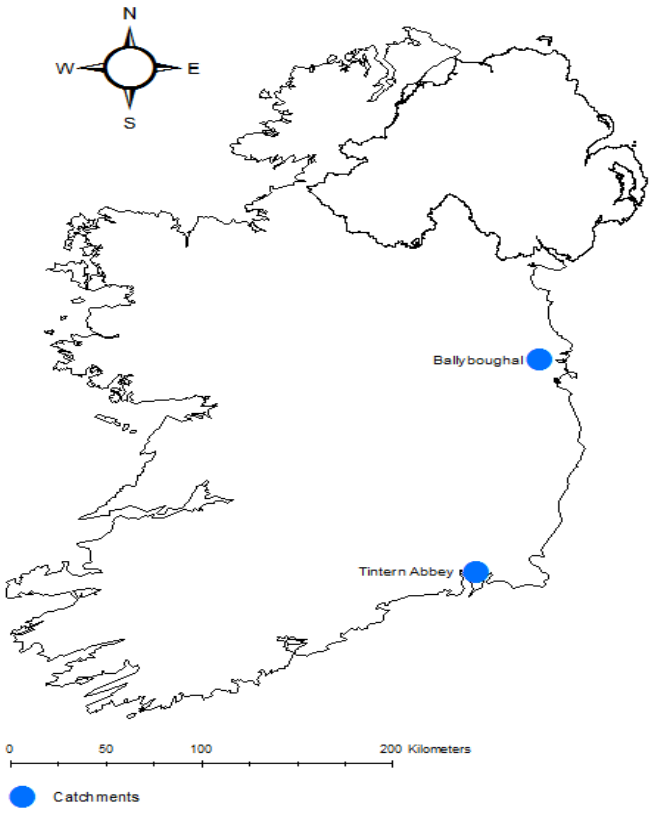

Figure 3.1 Locations of the Ballyboughal (BB) and Tintern Abbey (TTA) catchments in Ireland

Both catchments are used for pastures grazing or complex crop cultivation, and have down streams at medium to high level of eutrophication, based on the information provided by 2015-2017 Water Quality map (Environment Protection Agency of Ireland, 2018). Therefore, these two catchments were established as suitable locations to study the influences of agricultural catchment sediments on the water quality of streams and rivers. BB catchment has an area of about 23 km^2, and the soils include river alluvium, fine loamy drift with limestones, and siliceous stones. TTA catchment has an area of 10 km^2, with soils from river

alluvium, fine loamy drift with siliceous stones. In addition to farming activities on both catchments, there is a wastewater treatment plant in BB catchment. According to the EPA Geology map (Environment Protection Agency of Ireland), the TTA catchment is located over a poor aquifer, while BB catchment has bedrock that is considered a moderately productive aquifer. In each catchment, five locations are selected for sampling.

3.2 Sampling

Sampling took place between September 2017 and August 2018. Surface water samples were collected manually and automatically during events across locations on both catchments. Water samplers were placed at the outflows of both catchments to sample over high and low flow periods. The water samples were filtered, and subsampled for further analysis in the lab.

This thesis focuses on fluvial sediment at the outflows of the two catchments, which was gathered using time-integrated sediment traps, and deployed periodically to collect in stream suspended solids over a particular time period. The sediment traps are carefully removed from streams and emptied into clean 10 L containers which are kept as near as possible to the stream temperature while being transported back to the laboratory. The sediments are stored in a refrigerator and processed by wet sieving the fluvial sediment through 63µm steel stainless sieves, then centrifuged at 5000 rpm and the supernatant decanted. The sediments are then freeze-dried for further experiments and analysis.

Sampling seasons could be separated into “drought” season (from October to January) and “storm” season (February to September), which are prevalently used terms in Ireland. Based on the mean temperature and the mean precipitation data of the two catchments from the year 1981 to 2010 in the area of Ballyboughal and Tintern Abbey (Figure 3.2), it is clear that both catchments are rainy over the year; however, there are different patterns in rainfall with the mean monthly total precipitation in Tintern Abbey being much higher in Fall/Winter

(from October to January) than it in Spring/Summer (February to September), while the mean monthly total precipitation in Ballyboughal is more evenly distributed through the whole year, with slightly higher amount in Fall/Winter than it in Spring/Summer. In order to follow the prevalent terms and farming activities, sampling took places in events during the so-called "drought" season and "storm" season. Therefore, the events are distinguished by "drought" events and "storm" events. Besides, both of the catchments have moderate temperatures around the year, between 5 °C and 16 °C, with higher temperature in summer than in winter; Therefore, in order to compare both spatial and temporal differences between the two catchments, the samples for this study are collected at the beginning of October 2017 and at the end of February 2018, corresponding to "drought" event and the "flood" event.

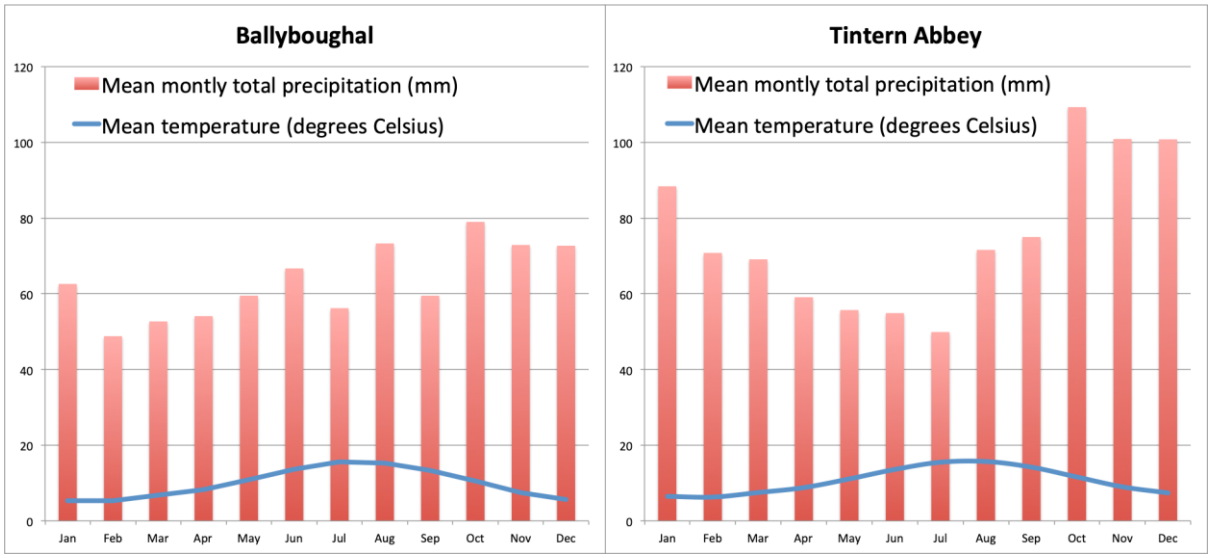

Figure 3.2 Mean monthly total precipitation (mm) and mean temperature (°C) of Ballyboughal and Tintern Abbey catchments from 1981 - 2010 (data retrieved from The Irish Meteorological Service (N/A))

3.3 Dual Cultural Diffusion Apparatus (DCDA)

The Dual Culture Diffusion Apparatus (DCDA) technique is an appropriate method to test the algal bioavailable P in suspended and particulate phosphorus (PP) (DePinto, Young, & Martin, 1981). The method used in this study was modified based on the studies of Deacon (2015) and Miller et al. (1978), with a scaled-up tank size. The apparatus consist of two

chambers, with each filled with 2 L of P-free algal growth medium; one is covered with foil and kept dark with sediments samples, while the other chamber is exposed to constant fluorescent light with P-starved algae (Figure 3.2). The method of making P-free algal growth medium is listed as Table A & B in Appendix. The chambers are separated by a 25 μm-thick Black Polycarbonate (PC) membrane with a pore size of 0.4 μm, which is smaller than the size of sediment samples (>0.45 μm). The algae specie used in this DCDA experiment is *Selenastrum capricornutum*, which is unicellular, has high growth rate, and is sensitive to toxicants, with cell sizes between 0.42 – 0.52 μm (Yamagishi, et al., 2017). Since the membrane size is smaller than both sediment samples and algae, it allows permeation of the dissolved nutrients for algae growth (such as NO_3^- and PO_4^{3-}) without mixing the algae and samples. The apparatus are agitated throughout the experiment; prevent adhesion of algae on the chamber. The agitation also allows the dissolved P permeates through the membrane. Additionally, the P-free growth medium in both chambers ensures that the only source of P utilized for algae growth within the system is the fluvial sediments. We should note that other nutrients, such as nitrite and bacteria, could also be the source for the growth of algae, but we are only tracking the P in algae before and after the DCDA experiment.

Figure 3.3 Dual Culture Diffusion Apparatus

The experimental procedure involves the addition of grinded freeze-dried suspended sediments samples to one reaction chamber and P-starved algae input pellets to the other. The algae input pellets are collected from an incubated algae culture, which have been "starved" with P for 9 days (Nover, 2004). The algal culture is separated by centrifugation at a speed of 5000 rpm with a sample-to-rotor center distance of 4 cm. The combined precipitations from the centrifuged algae culture are used as the input algae pellets. Extra input pellets are created to estimate the initial P concentration into the system. The input pellets are put into the bright chamber, and would be harvested after 3 days. The algal cells in the 2L medium is also separated by centrifugation at a speed of 5000 rpm, and all the precipitations are combined into the harvested pellets. The harvested supernatant is poured back into the chamber as it might contain dissolved P that has not been consumed. Both input algae pellets and harvested algae pellets must be diluted with deionized water to 50 mL, digested using $K_2S_2O_8$ solution, and then the TP concentrations of algae are analyzed by using colorimetry. The total phosphorus (TP) concentration of the harvested algae (C_{t1}) is subtracted from the TP concentration of the input algae three days earlier (C_{t0}), which is considered as the bioavailable P that has been utilized for algae growth within the three days. The graph of accumulated bioavailable P that has been continuously utilized through algae growth is generated at the end of the experiment (~25 days). The experiment continues until algae growth has consumed all the bioavailable P from the sediments. The duration should not exceed 30 days based on the concentration of P in the sediments (Lambert, 2012; Miller, Greene, & Shiroyama, 1978; DePinto, Young, & Martin, 1981). The experimental procedure sufficiently estimates the amount of bioavailable P within the suspended sediments, and tracks P releasing kinetics from sediment samples. Furthermore, following completion of the DCDA experiment, sediment samples are recollected; freeze dried, and subjected to further solid phase P analyses using multiple techniques.

The harvested algae and input algae are digested by using a digestion reagent that was made of 60g of $K_2S_2O_8$ and 100 mL of 1.8M of H_2SO_4 in 1 L of Milli-Q water, which convert the P in algae into inorganic P, and is measured in P-PO_4 by using molybdate colorimetric method (Worsfold & et al., 2005):

$$PO_4^{3-} + 12MoO_4^{2-} + 27H^+ \rightarrow H_3PO_4(MoO_3)_{12} + 12H_2O$$

$$H_3PO_4(MoO_3)_{12} + \text{reducing agent} \rightarrow \text{phosphomolybdenum blue [Mo(VI) } \rightarrow \text{ Mo(V)]}$$

Even though the digestion is considered to be sufficient, but the colour formation sensitivity could be highly affected by the $[H^+]/[MoO_4^{2-}]$ (ideally at a ratio of 70) and pH (Worsfold & et al., 2005). In this study, the pH was set to 7 at the beginning the DCDA experiment, while no pH adjustment was made during the DCDA. Therefore, the recovery of digestion and the pH adjustment for the samples should be considered as limitations in DCDA method. Because of the time limit, DCDA experiment was performed only for four samples (two events for two locations), without repetition.

3.4 Chemical Sequential Extraction (CSE)

Chemical sequential extraction of phosphorus is a common technique to distinguish operationally defined P fractions within sediment samples. The method applied in this study was developed by Psenner (1984), and modified by Hupfer et.al (2009). The chemical sequential extraction scheme for P in this study yielded the following operationally defined P fractions: loosely-bound P (Loose-P), iron/manganese-bound P (Fe/Mn-P), aluminum oxide-bound P (Al-P), organic P (OP), Ca-P, and other P. The detailed procedures are listed in Table 3.1.

Table 3.1 Modified chemical extraction procedure

Extractant/fraction	Time/ Temp.	Expected P species
Milli-Q water	2 h, ~25°C	Loose-P
0.1M $NaHCO_3$ 0.11M $Na_2S_2O_4$ (BD solution)	1 h, ~25°C	Fe/Mn-P

0.1 M NaOH	16 h, ~25°C	Al-P
1 mL 0.1 M NaOH extract + 0.25 ml 0.5 M HCl + $K_2S_2O_4$ digestion reagent	1 h, 120°C	P fraction from this step – Al-P = OP
0.5 M HCl	1h /~25°C	Ca-P
1.0 M HCl	Combust 550°C, 8 h. 1M HCl, 120°C, 1 h	Other-P

The samples are freeze-dried and finely grinded before the CSE. About 0.1 ~ 0.15 g of samples are used, and each sample was done in duplicate. The original chemical sequential extraction procedure involves centrifuging liquid extracts from the sediment residues under 5000 rpm after each step. However in this study, to prevent the loss of residues when pouring off supernatant between each extraction, an apparatus that consists of vacuum filtration manifold and reaction vials with filters was used (Figure 3.3). The filters used during extraction processes are track-etched membrane with a pore size of 0.4μm, which are phosphorus free, non-destructive and reproducible under either acidic or basic conditions (Apel, 2001). The apparatus is cleaned thoroughly each time after use to avoid any contamination during the extraction process.

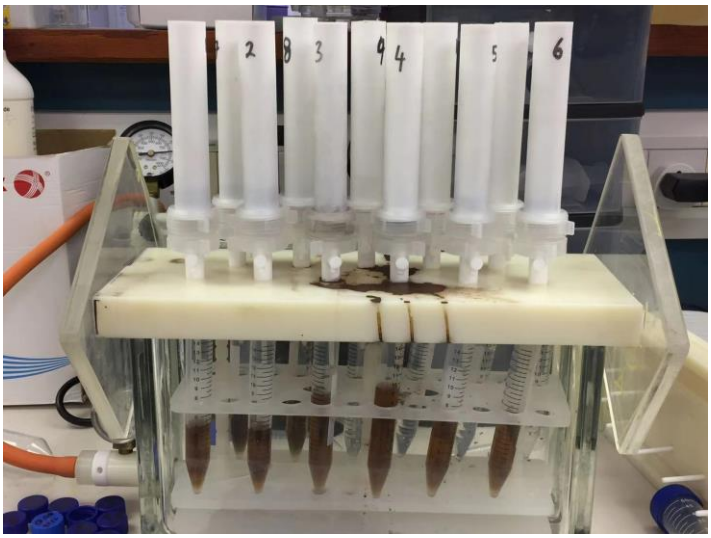

Figure 3.4 Extraction Apparatus

Time between collection of the supernatant and analysis is kept to a minimum. The extracted solutions are analyzed using molybdate colorimetry on a nutrient analyzer to measure the concentrations of P in the form of P-PO_4. Chemical sequential extraction will provide distinction between P fractions in addition to a basic understanding of the P speciation in sediment samples.

3.5 X-ray Fluorescence (XRF) Spectroscopy

XRF spectroscopy is measured using the soft X-ray microcharacterization beamline (SXRMB) at the Canadian Light Source. The samples are finely grinded, and spread evenly on the carbon strips, which could be used for both XRF spectroscopy and XANES spectroscopy. A photon-energy of 7200 eV is used to excite the fluorescence signals and the XRF was recorded at a range of energy from 1500 to 7200 eV. Since these sediments samples are all fine powders with similar chemical composition (matrix), it is reasonable to assume they all scatter similarly. Thus each XRF spectrum is normalized in intensity using the scattered X-rays (off the sample with an energy close to that of the incident X-ray – 7200 eV). By comparing the XRF results for pre- and post- DCDA samples from different catchments, it is possible to semi-quantitatively track the change of elemental concentrations before and after the DCDA, thus providing a general understanding of P speciation and transformation at an elemental level.

3.6 X-ray Absorption Near-edge Structure (XANES) Spectroscopy

XANES spectroscopy is also conducted at the SXRMB beamline of the Canadian Light Source. P-K edge, Ca-K edge, and Fe-K edge spectra for all samples are generated, and cross-compared to make a better understanding. A 7-element Si-drift detector was used to measure the fluorescence yield of low concentration samples, which the sample drain current was used to record the total electron yield of highly concentrated samples, such as references.

The samples are scanned at least twice, and the spectra are processed by Athena software (Ravel & Newville, 2005). The duplicated scans compared to the original spectrum to check the reproducibility (usually identical to each other), and then merged into an average spectrum followed by normalization. The normalization of references and samples spectra allows the identification of main chemical components by fingerprinting. After the identification of dominant compounds, a linear combination fitting (LCF) analysis is applied to estimate the fractions of the identified compounds in each sample.

XANES spectroscopy coupling with Chemical Sequential Extractions are applied to fluvial sediments pre- and post DCDA experiments to investigate P transformation mechanisms and processes related to fluvial suspended sediments. Combining both chemical sequential extractions and XANES analyses in this study provides comparison results of the two techniques, and gives comprehensive understandings of P speciation.

3.7 Statistics

Ideally, all the experiments should be performed in triplicate for improved statistics and quality control. In this study, the chemical extraction for each sample was done in duplicate, and the XANES spectroscopy for each sample was scanned at least twice. Due to the limitation of time, DCDA experiments had only been performed once. Even though the reproducibility of DCDA experiments cannot be confirmed, it is still quite useful in studying P transformation as the bioavailable P in post-DCDA samples had been consumed. To ensure the precision of the P measurement of algae digestion, the algae pellets were first diluted to 50 mL, and multiple digestions were performed to ensure precisions of the results, the average values were used in final calculations.

The samples were duplicated in CSE experiments. The results were averaged and the standard deviations of the results were calculated to examine the reliability of the duplicates.

Similarly, the XANES spectroscopy was also performed twice for each sample (mostly identical to each other), and the average was used for both fingerprinting and LCF analyses.

When measuring the P concentrations using colorimetry, a calibration was performed before each batch of measurement, and only the calibrations with R-square values higher than 0.995 would be accepted. Besides, control samples, such as blanks and known samples, were measured before, during, as well as after each batch, for quality checks. Based on the calibration results, it is found that the results are less reliable when the P concentration is less than 10 ppm (i.e. 0.01 mg/g).

XRF scans were performed once for each sample, since the trends of elements were compared semi-quantitatively, instead of absolute values, it is considered to be acceptable.

For XANES analyses, every sample was scanned twice, checked for reproducibility and merged for normalization. However, the accuracy of linear combination fitting (LCF) could be highly affected by baseline corrections and edge-step normalization procedures, the error is usually estimated to be 10% (Gu, et al., 2019), or as large as 17% (Werner & Prietzel, 2015). Thus P fraction value smaller than 5% according to LCF analysis of XANES data is not considered to be reliable, and needs to be confirmed (Werner & Prietzel, 2015). These limitations of XANES highlight the importance of the conjunctive use of multiple methods in studying P speciation.

4 Results and Discussion

4.1 Bioavailable Phosphorus in Fluvial Sediments Samples by DCDA

The drought and flood events in Ballyboughal catchment are named as BB Oct_17 and BB Feb_18 respectively. The drought and flood events in Tintern Abbey catchment are named as TTA Oct_17 and TTA Feb_18 respectively.

Figure 4.1 shows the accumulative bioavailable P results from DCDA experiments. The algae growth experiments continued until algae stop consuming P, i.e. all the bioavailable P are used up in about 20 days. Table 4.1 records the pH of the original P-free algal growth medium for each suspended sediment sample.

Table 4.1 pH values for the P-free algal growth medium of each DCDA batch

DCDA Batch	TTA Oct_17	TTA Feb_18	BB Oct_17	BB Feb_18
pH	7.25	7.28	7.31	7.29

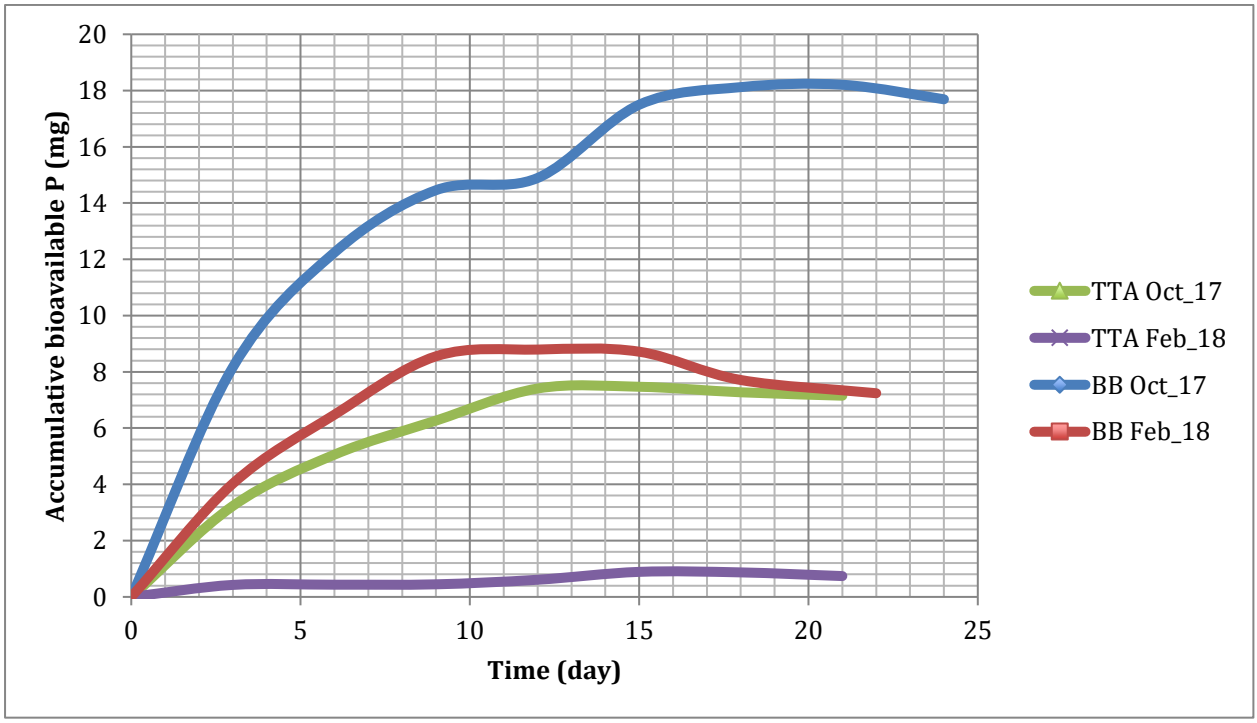

Figure 4.1 Accumulative bioavailable P (mg) of fluvial sediments (0.4 g) from TTA and BB catchment

The accumulative bioavailable P results show that there is a difference in total bioavailable P between catchments, where samples from BB catchment produce more bioavailable P than TTA when all experiments are using the same amount of sediment samples. When comparing the seasonal differences within catchments, it is clear that samples from both catchments produce more bioavailable P during drought events (i.e. Oct 17). The rates of accumulative bioavailable P suggest the same trend as the total bioavailable P, that samples from BB catchment have higher releasing rate at beginning of the experiment (i.e. 2.72 mg/day for BB Oct_17, 1.34 mg/day for BB Feb_18, 1.08 mg/day for TTA Oct_17, 0.14 mg/day for TTA Feb_18), and the P transformations in drought events are faster within the same catchment. It needs to be noticed that the P releasing rates do not necessarily represent the real releasing rates on the actual field sites, as the experimental temperature is around 20 °C, which is higher than the local temperatures at the sampling site (Figure 3.2). Considering cultivation and pasture grazing are the major agriculture activities in both of catchments, the spatial and temporal differences suggest different transformation mechanisms. Correlating the DCDA results to the geographic information that BB catchment has an area of 23 km^2, which is over twice as the area of TTA catchment (10 km^2), the fact that the bioavailable P in the sediments of BB outflow is more than double of it in TTA catchment can be explained by the accumulations of P from the whole area of catchment. Hence, larger catchment might have more accumulated bioavailable P, and would have greater impact on the downstream water quality. In addition to the area sizes of the catchments, the wastewater treatment plant on BB catchment is another major reason that contributes to the huge bioavailable P in the suspended sediments from the BB outflows, as there still would be almost 10% of the organic matter remained in the effluents from wastewater treatment plants, which could contain high concentration of P (Kim, et al., 2011).

4.2 Phosphorus Fractionation in Fluvial Sediments Samples by CSE

Following DCDA experiments, sediments were recollected and analyzed for comparison with the fluvial sediment P compositions prior to commencement of the DCDA experiments (Figures 4.2 and 4.3).

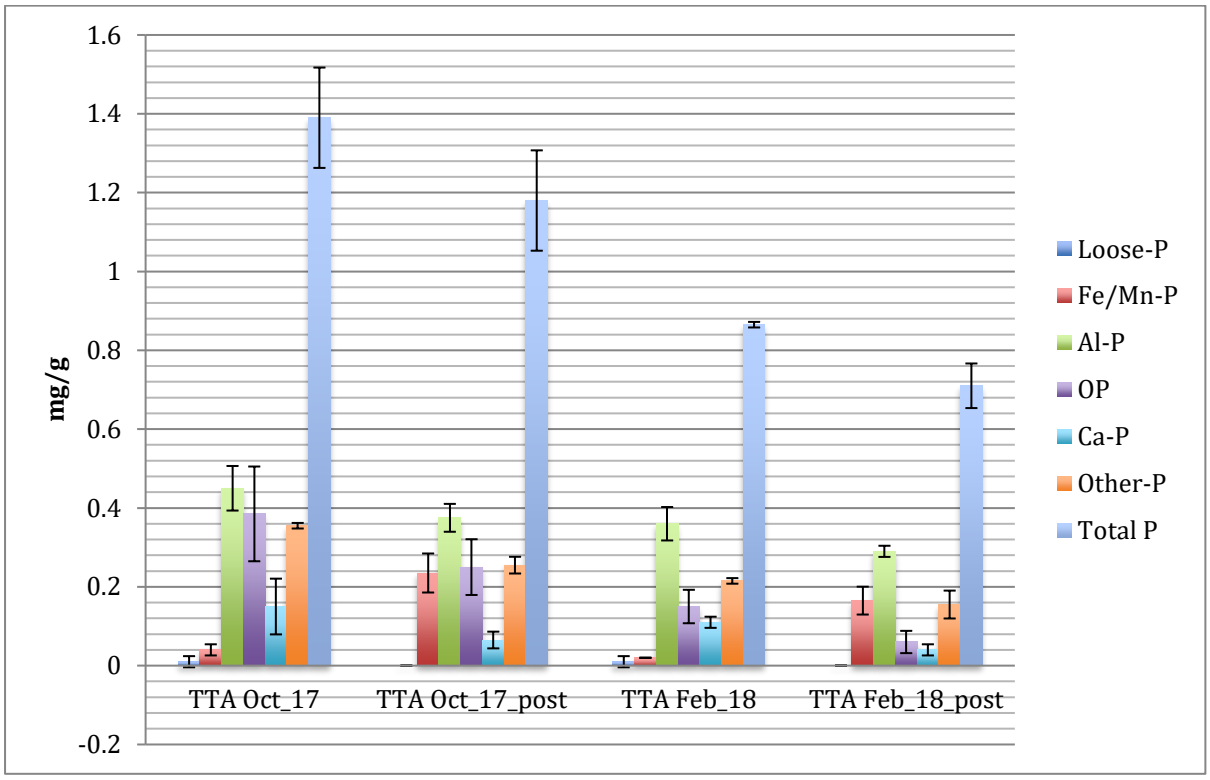

Figure 4.2 P fractions in the fluvial sediments of the TTA catchment stream pre- and post- DCDA experiments (mg/g dry weight). Error bars represent the standard deviations of the duplicates from the mean values

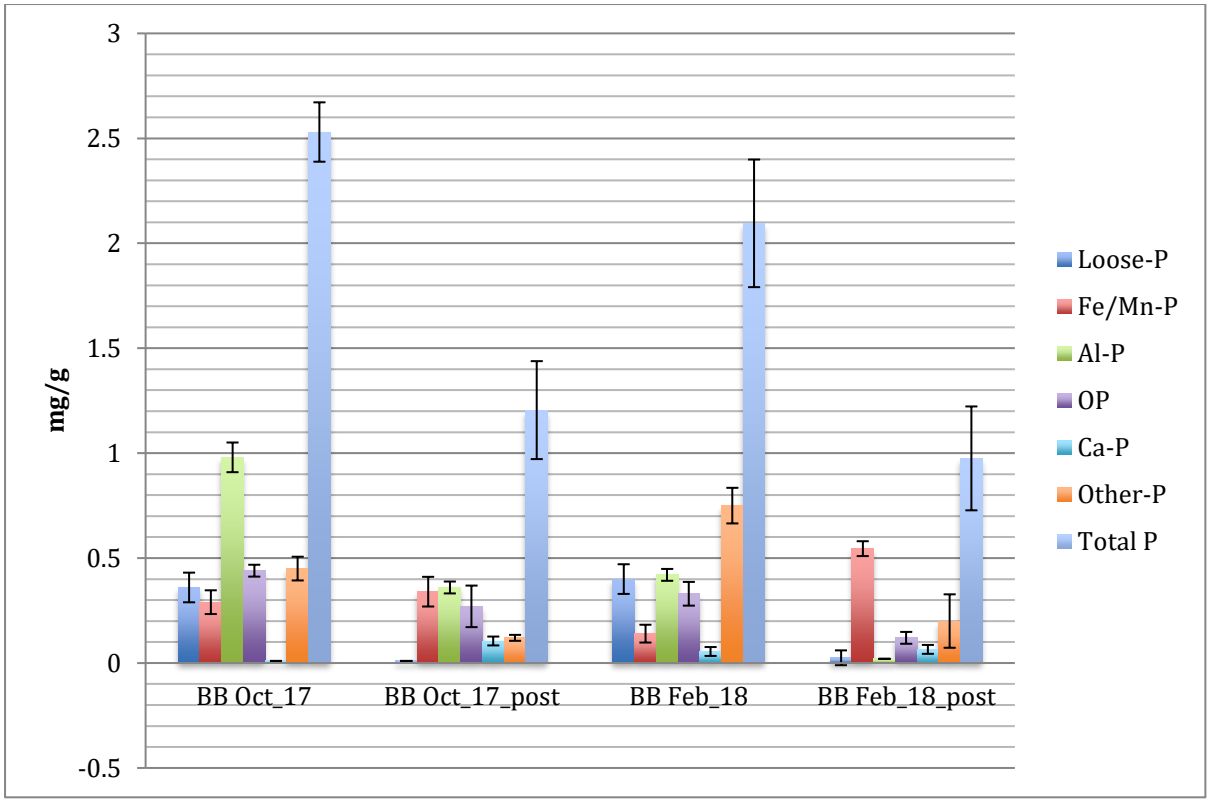

Figure 4.3 P fractions in the fluvial sediments of the BB catchment stream pre- and post- DCDA experiments (mg/g dry weight). Error bars represent the standard deviations of the duplicates from the mean values

The raw CSE data can be found in Appendix (Table C). The error bars in Figure 4.2 and Figure 4.3 represent the standard deviation of the duplicate values to the average, which are rather small compared to the average values, indicating the reproducibility of the CSE experiments and the validity of the CSE results.

The CSE results show that the TP amounts of pre-DCDA sediments from BB catchment are higher than the sediments from TTA catchment for both events, while TP amounts of the post-DCDA sediments from both catchments are rather close for both events, which means that the sediments from BB catchment lost more TP in DCDA experiments than those from TTA catchment. This trend confirms the accumulative bioavailable P results from DCDA experiment that BB catchment releases more bioavailable P in general. Hence, higher amount of TP is related to higher potential of bioavailable P transformation. Since there are more farming activities in Spring/Summer than those in Fall/Winter, as a result of excessive

application of fertilizers during drought season, both of the DCDA and CSE results agree that the TP load and bioavailable P amount are higher for samples in Oct_17 than those in Feb_18.

For both catchments, the reduced concentrations of the loose-P, and part of Al-P, OP and other-P in samples after DCDA experiments suggest these forms of P are more easily bioavailable. Interestingly, the fractions in these two catchments show a difference in Ca-P change that it increased in BB but decreased in TTA. Based on the geological information of the two catchments that the soils in BB contain significant calcite (Environment Protection Agency of Ireland), and the higher pH of BB outflow (Table 4.2), the different results of Ca-P changes on the two catchments can be explained by the effects of pH on the $CaCO_3$ associated P ($CaCO_3$-P). Previous studies have shown that in both soils and marine sediments, $CaCO_3$-P is more sensitive to pH change, i.e. $CaCO_3$-P dissolution is easily driven by proton release than other types of Ca-P (e.g. apatite) and organic P bound with secondary minerals (Fe/Al oxides) (Zhang, et al., 2020; Kraal, et al., 2017). The CSE results of this study proves that this interpretation is also true in fluvial sediments, as the increased Ca-P after DCDA experiments is associated with higher pH of outflow from BB catchment. Regarding the Fe/Mn-P, all the samples show an increased potion after DCDA experiments. Previous studies suggest that iron (III) oxides are able to sorb dissolved organic matters, and recrystallizing the complexes to more stable complexes (Eusterhues, et al., 2014; Hansel, et al., 2005), hence stablize sediments from biodegradation. In this study, chemical extraction results after DCDA experiments suggest increased Fe-P fraction reduces the P bioavailability of the sediments, with the potential to sequester P released from soils, which may potentially buffer downstream surface water quality degradation. Based on previous studies, concentrated HCl mainly extracts stable organic P and complex Ca-P (Ajiboye, 2007; Zhang, et al., 2020), which indicates that the other-P potion in this studies consist of stable OP and Ca-P. Compare to loose-P, only part of Al-P, OP, and other-P are transformed to bioavailable

P. This trend indicates the existence of stable complexes in these fractions, just as some studies suggested that those primary minerals associated P can easily become bioavailable by weathering, while the secondary mineral oxides promote P retentions in the sediments (Zhang, et al., 2020). Therefore, analyses at molecular level are required to provide in-depth understanding of the P speciation.

These results indicate seasonal dynamics in P bioavailability associated with fluvial suspended sediment with elevated P bioavailability following summer low flow periods and droughts (Figure 3.2, 4.2, and 4.3). This conclusion can be confirmed by comparing the dissolved P in water outflow shown in Table 4.2, which shows that dissolved P in outflows has contrary behavior regarding drought/storm seasons in each catchment, meaning that more P are washed off to water body during high flow. Correlating the results with the temperature and rainfall differences between the two catchments, it is evident that the P speciation in both outflow and sediments are affected by the weather and farming activities. Therefore, in order to improve the downstream water quality, it is important to keep monitoring the fertilization activities during drought season and prevent sudden elevation of P during storm events. Also, considering that the decrease of pH may induce more dissolutions of $CaPO_3$-P in the BB catchment, it is critical to monitor both farming activities and pH changes in this area under abnormal temperatures, as lower pH in water bodies is a normal phenomenon because of more uptakes of greenhouse gases, such as under high temperatures (Kraal, Kijkstra, Behrends, & Slomp, 2017).

Table 4.2 Dissolved P data and average pH for all catchment outflows

Water Sample	BB Outflow Oct_17	BB Outflow Feb_18	TTA Outflow Oct_17	TTA Outflow Feb_18
Dissolved P in PO_4^{3-} (mg/L)	0.12	0.17	0.08	0.12
Average pH	8.55	8.79	7.27	7.42

4.3 Elemental analysis of sediments by XRF

In order to understand the P bioavailability and transformation, we study the elemental distributions of the sediments before and after the DCDA experiment using the XRF. The XRF spectrum (Figure 4.4) is normalized to the same intensity of the scattered X-Ray (7200 eV, which is the photon energy used to produce XRF). Therefore, the XRF counts of each sample are comparable in terms of relative intensities of different elements. It should be noted that the excitation energy of 7200 eV was selected to study the higher energy elements, such as Ca and Fe, which are important elements associated with P transformation. At this energy, the XRF intensity of light elements, such as Al and P, is rather low ,due to either the low concentration or low SRF efficiency. Thus, in this XRF study, it was chosen to focus on the higher energy elements. The graphs in Figure 4.4 demonstrate the presence of Fe (~6390 eV), Ca (~3690 eV), and Mn (~5890 eV).

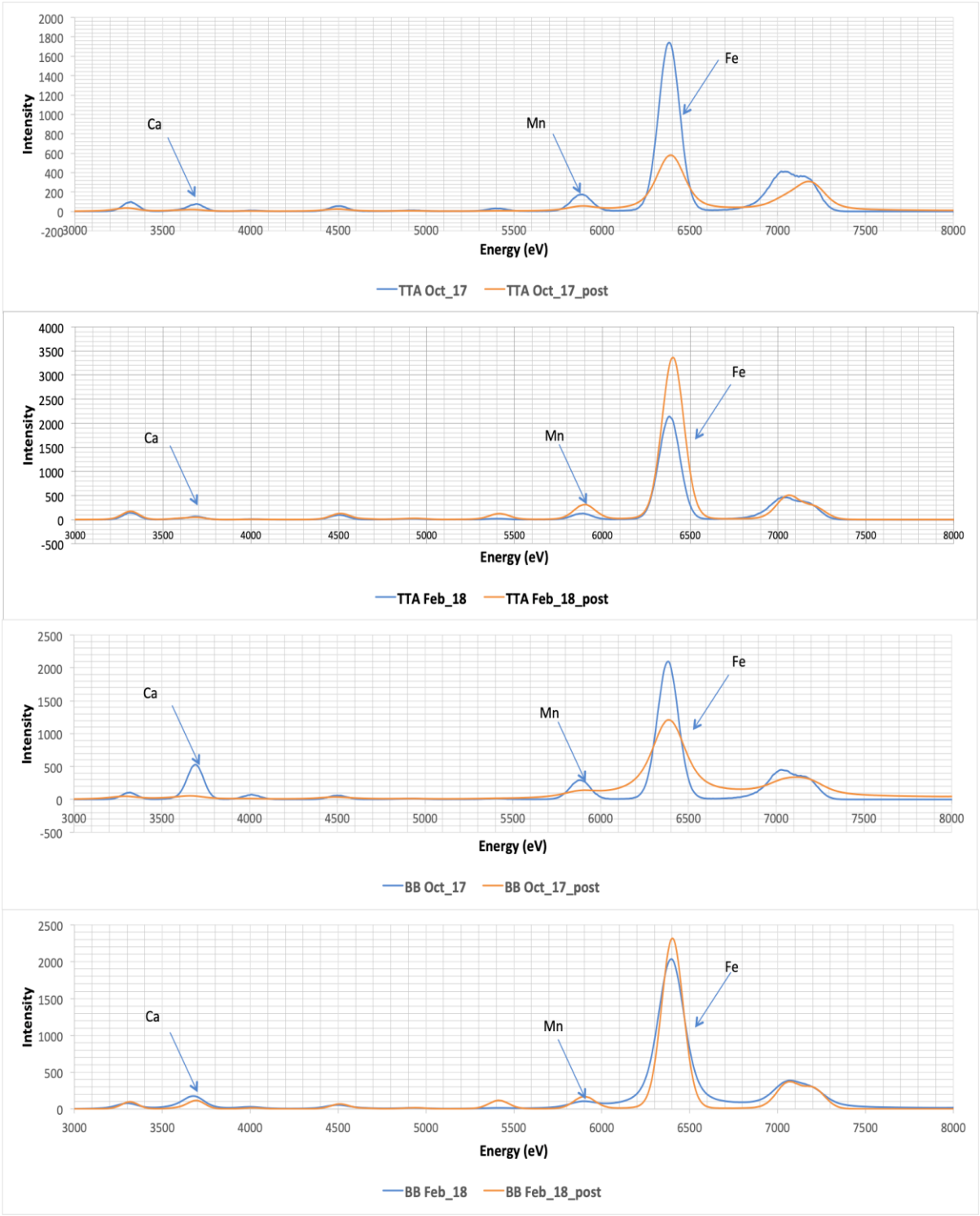

Figure 4.4 XRF graphs for the fluvial sediments of TTA and BB catchments pre- and post- DCDA experiments

The results show that all samples are high in Fe. While the original samples all have similar Fe intensity, the change of Fe for post-DCDA samples is quite different. In general, the amounts of Fe changes in TTA are larger than those in BB, which agrees with the CSE

results (Figure 4.3 and 4.4), suggesting a more active role that Fe plays in the P biotransformation of TTA sediments. After DCDA experiments, the amount of elemental Fe increased in flood events (Feb_18) for both catchments (Figure 4.4), which is in agreement with the increased Fe-P in these samples as revealed by the chemical extractions (Figure 4.2 and 4.3). However, there is significant decrease in the amount of Fe in drought event (Oct_17) for both sites, much more so for the TTA, after the DCDA experiment (Figure 4.1), which is contradictory to the accumulation of Fe-P, according to the chemical extraction result (Figure 4.2 and 4.3). These observations suggest differences of the Fe compositions in each fluvial sediment site/event, as XRF is only able to provide the elemental information of samples. For example, it should be noted that there is hardly any Fe-P in the TTA sediment before the DCDA experiment, thus the increase of Fe-P as a result of DCDA is likely because of the interaction of Fe and organic-P in the TTA samples, regardless of the amount of elemental Fe. The changes of Mn concentrations of fluvial suspended sediments have similar trend to Fe concentrations.

The Ca XRF intensities in original samples indicate that the samples from BB have more Ca concentrations than TTA in general, which agrees with the geological composition of the soils from BB catchment which shows a high limestone content. The Ca concentrations of all samples generally decrease after DCDA experiments, with a large drop for the drought season (Oct_17) at the BB site. This significant loss of Ca at the BB site is not related to the increased Ca-P as observed by CSE (Figure 4.3), rather due to the chemical form of Ca in BB site (e.g. more loss of soluble Ca during the DCDA experiment). For TTA, which has much lower amount of elemental Ca compared to BB, the decrease in elemental Ca is consistent with the decreased Ca-P shown by the chemical extraction method (Figure 4.2). The geochemical analysis of suspended sediments using XANES is performed in order to better

understand/compare the trends of Ca and Fe with respect to the revealed changes using chemical extractions and XRF of Ca-P and Fe/Mn-P.

4.4 Chemical analysis of sediments by XANES

Figure 4.5 shows P K-edge spectra of selected reference compounds in agricultural soils and sediments. $FePO_4 \bullet 2H_2O$, Phytic Acid, and $Ca_2(PO_4)_3$, can represent for Fe-P, OP, and Ca-P, respectively. The reference spectra selections are based on the presences of compounds in natural agricultural environment, as well as comparions with the sample spectra. The selected references spectra have some characteristics features that are unique: the spectrum of $Ca_2(PO_4)_3$ has a broader post-edge "shoulder" around 2155 eV, and a small resonance peak at post-edge at the energy of 2162 eV; the distinguished feature of $FePO_4 \bullet 2H_2O$ is the peak at pre-edge around 2149 eV (as indicated by the dotted lines), and the peak is right-shifted; the spectrum of phytic acid is quite "smooth" compared to other spectra, and does not have any distinguishable feature. The reason that phytic acid can be considered as a representative of organic-P is that most of the XANES spectra of OP are quite similar, and cannot be distinguished by P-K edge (Ajiboye, 2007), which shows the insensitivity of organic chemicals for P K-edge XANES.

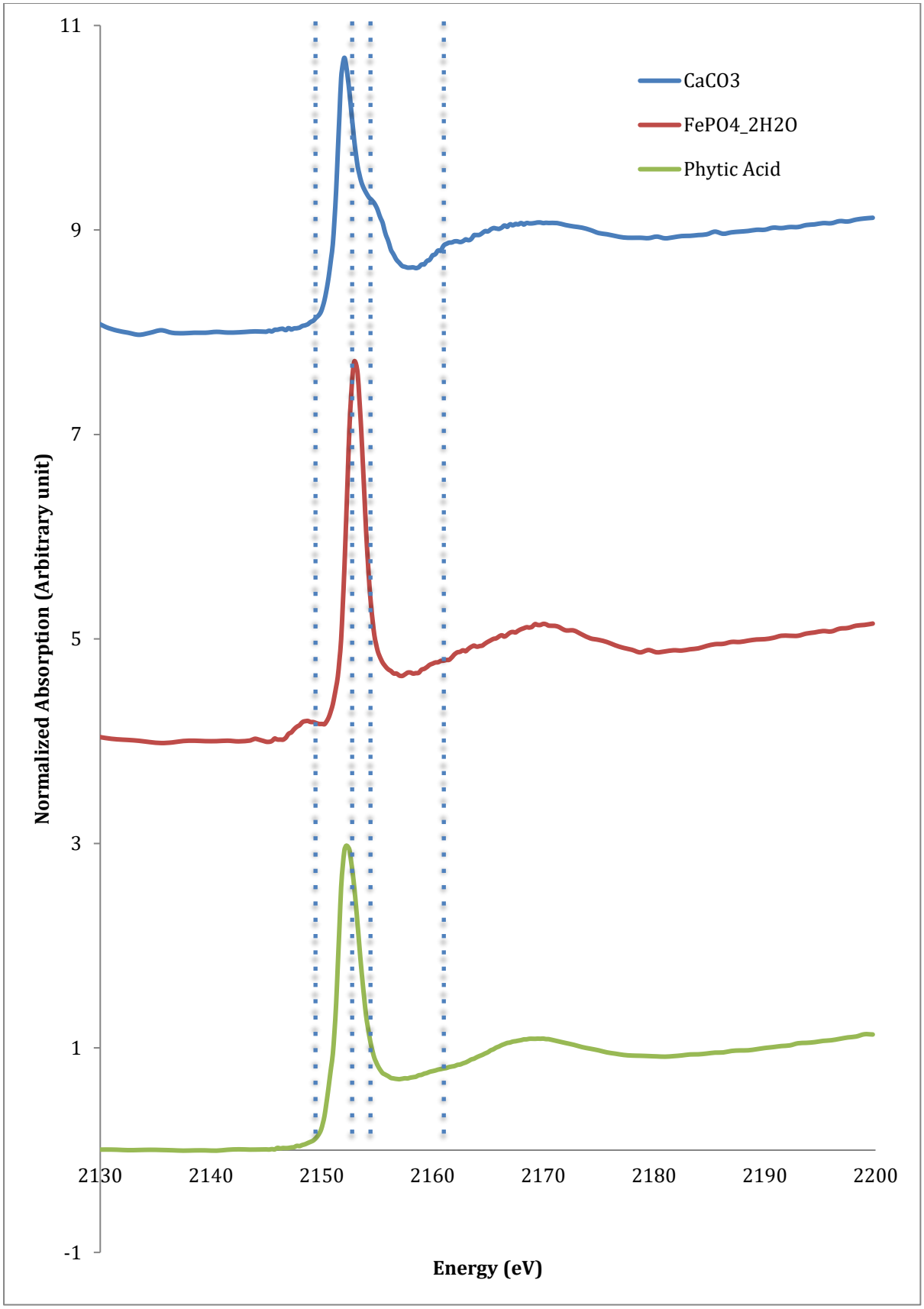

Figure 4.5 Normalized P K-edge XANES spectra for reference P compounds

Figure 4.6 includes the P K-edge XANES spectra for all the samples and corresponding LCF results. All the samples have similar patterns, indicating the existence of similar P related compounds in these samples. All samples have small elevations at 2149 eV and right-shifted peaks, indicating the presence of Fe-P; the spectra are generally smooth, which indicates that OP is the major component in samples. Some of the samples have broader post-edge "shoulder" at 2155 eV and scatters at 2162 eV, suggesting the presence of $Ca_3(PO_4)_2$. The LCF lines with each corresponding R-factor values are presented in Table 4.3. The smaller R-factor value indicates a better fitting result. It is noticeable that all of the fittings have small R-factor values (<<0.05), which means that the sediment samples are well represented by the selected references. The detailed P fractions are provided by LCF compositions (Figure 4.7).

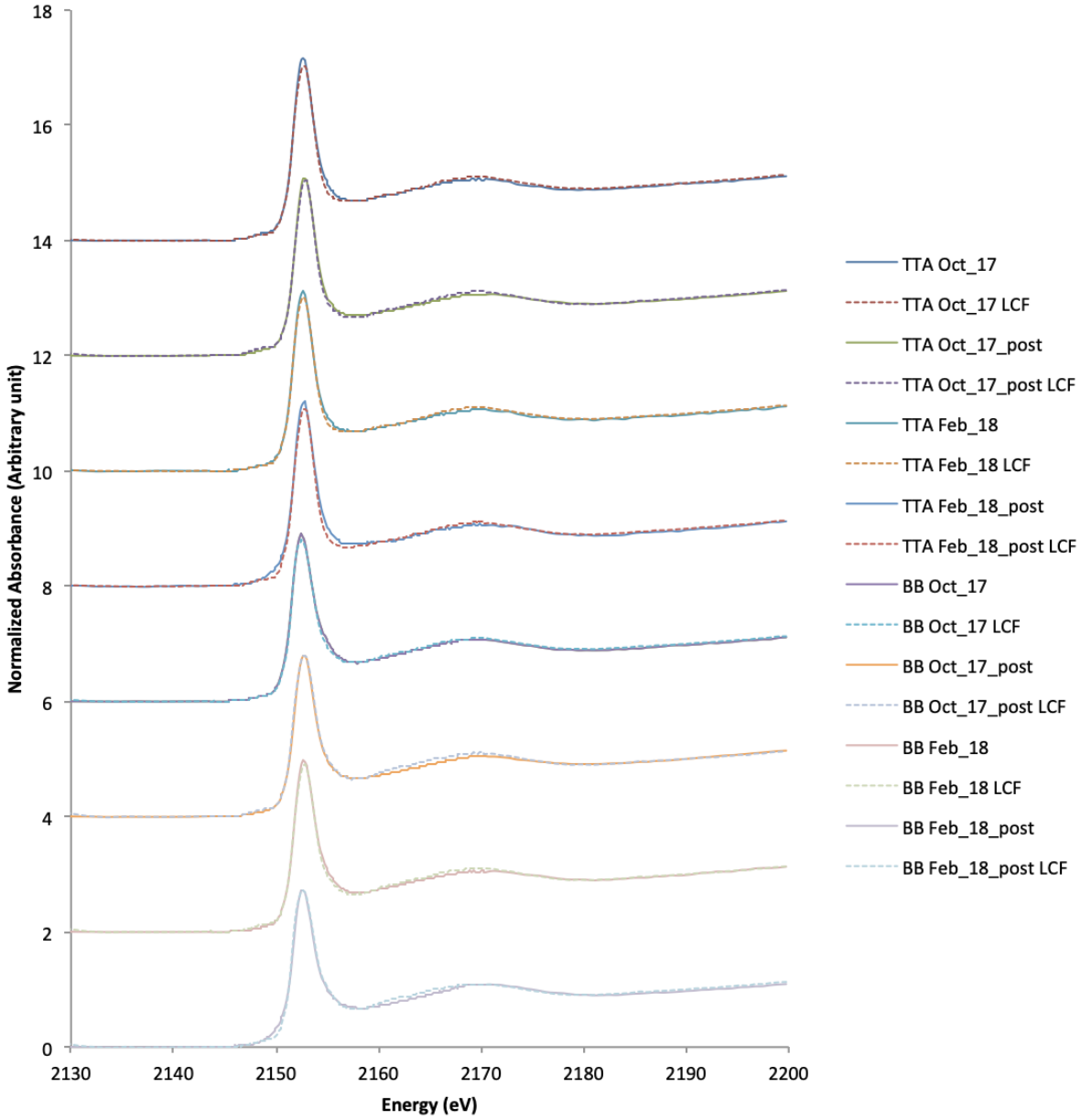

Figure 4.6 Normalized P K-edge XANES spectra and LCF spectra for the fluvial sediments of TTA and BB catchments streams pre- and post DCDA experiments, the spectra were offset for a better comparison

Table 4.3 R-factor values for each P K-edge XANES LCF fitting to the original spectrum

Sample	TTA Oct_17	TTA Oct_17_post	TTA Feb_18	TTA Feb_18_post	BB Oct_17	BB Oct_17_post	BB Feb_18	BB Feb_18_post
R-factor	0.0047	0.0038	0.0030	0.0019	0.0026	0.0066	0.0048	0.0063

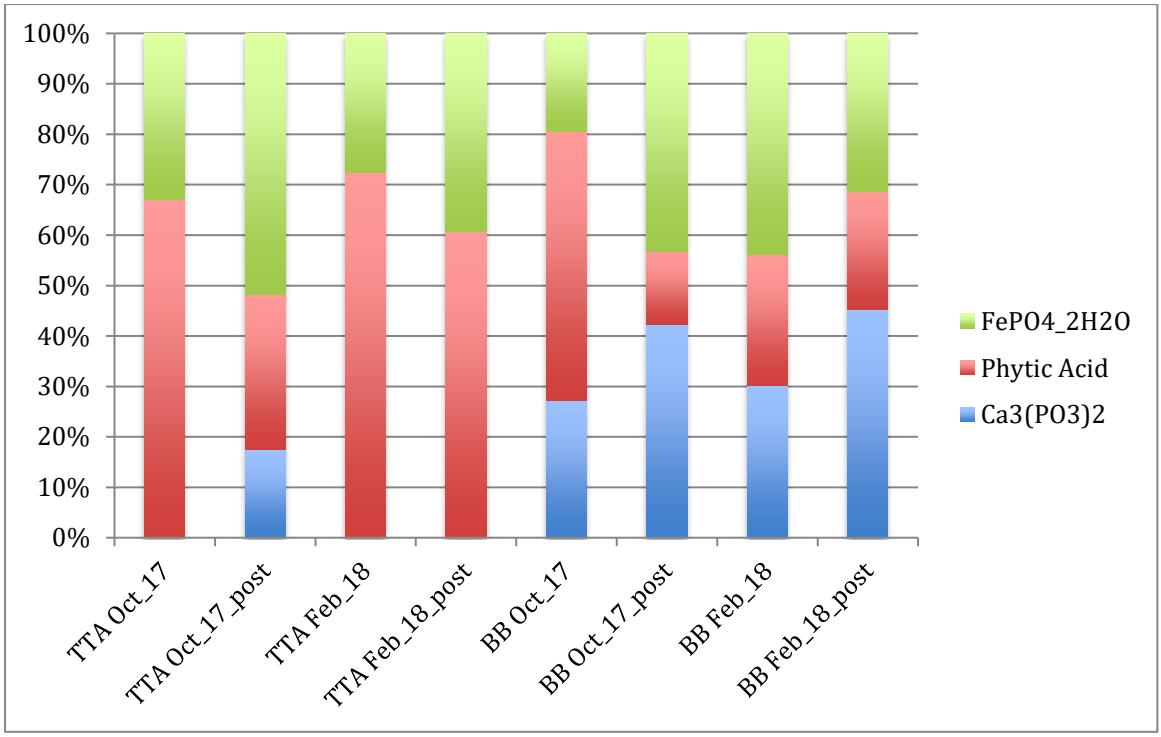

Figure 4.7 LCF composition results for P in samples

The LCF fraction results present a decline of phytic acid in all of the the samples after the DCDA experiments, which is in agreement with the CSE results. Ca-P is generally higher in BB than it in TTA, which agrees with XRF results and the given geological information that the soils in BB catchments consist of limestones. In agreement with the CSE results, there is also a slight increase in Ca-P in the BB site after the DCDA, indicating of the accumulation of Ca-P as a result of P bio-transformation. Samples from TTA are generally higher in organic-P, and reduced after the DCDA experiment, in agreement with the loss of organic-P as a result of algae growth. $FePO_4\_2H_2O$ fractions increased after DCDA experiments except for the fluvial sediment collected from BB stream in February 18, which is also consistent with the increase of Fe-P as shown by the CSE. While Organic-P fractions declined after DCDA experiments, $Ca_3(PO_4)_2$ fractions all increased, which does not follow the change of chemical extractions. The descripacy might due to that part of the loose-P and other-P also contains Ca-P in chemical extractions (Ajiboye, 2007). Besides, phytic acids can easily bond to metal ions such as Fe and Ca, and present in soils as manures (Graf, 1983),

which means that there are parts of the phytic acids bonding with metals and are hard to be separated. Therefore, the descripacies between P K-edge XANES and chemical extractions are due to the chelating of phytic acid and humic metals. The generally "smooth" features of nearly all samples suggest that OP dominates the P fractions, and the fingerprinting technique shows the presence of Fe-P and Ca-P in samples, especially in post-DCDA samples. These results indicate that metals bond to organic matters in sediments, which could limit samples from biodegradation.

Another descripancy of the XANES with CSE is that XANES did not show the presence of Al-P in samples. This difference could be due to the extraction of the Al associated compounds by CSE are not necessarily related to P, or the lack of distinct feature of the P K-edge of Al-P reference (Ajiboye, 2007). Thus Al-P is not included as a reference for LCF analysis. Considering that a certain compound should takes more than 5% of the portion in a sample to show the distinguishable feature in XANES (Werner & Prietzel, 2015), it suggests that there is no significant amount of Al-P in these samples.

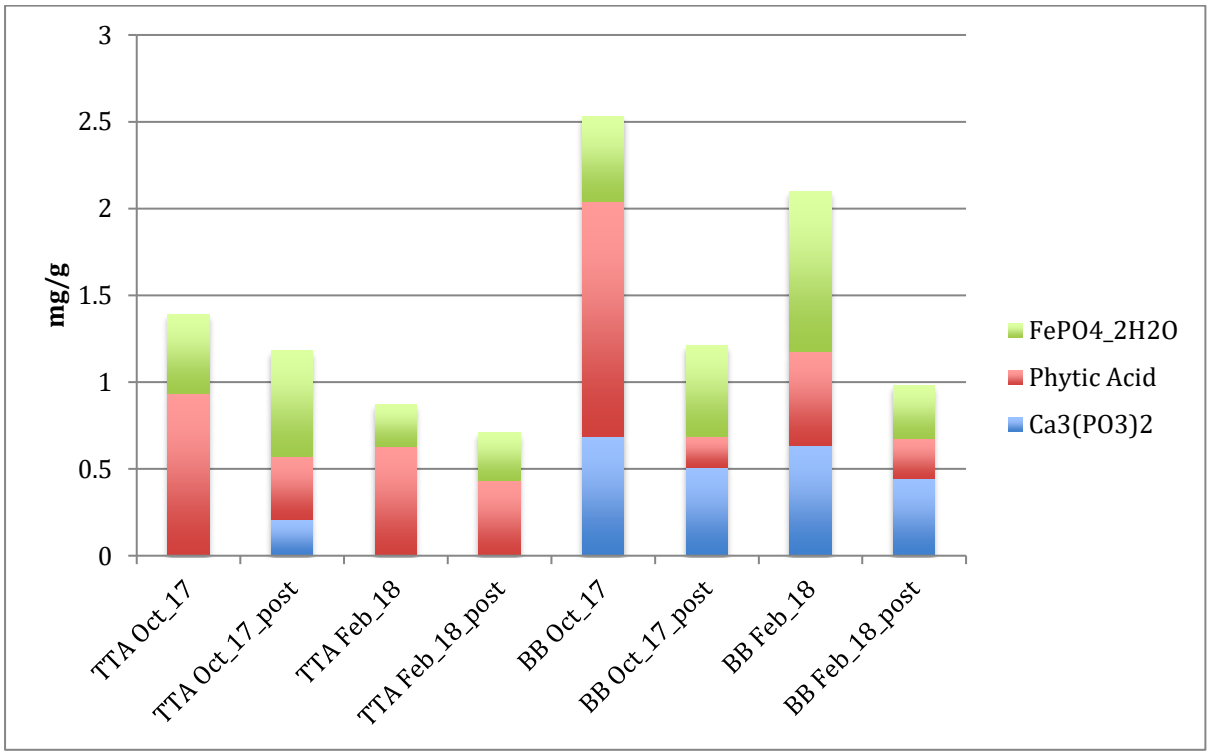

Figure 4.8 P concentrations (mg/g) of samples based on LCF compositions results

In addition to the P fractions generated by LCF, detailed P concentrations of samples based on the TP values from CSE and compositions from LCF are also shown in Figure 4.8. The P concentration changes in Figure 4.8 show that the amount of OP decreased after DCDA experiments. While there is no clear pattern in changes of $Ca_3(PO_4)_2$ and $FePO_4\_2H_2O$. It is obvious that the inorganic potions account for more in TP after DCDA experiments. The changes confirm that OP are more likely bioavailable, and metal ions helps prevent sediments from biodegradation. Therefore, Ca and Fe K-edge XANES spectra are generated to further examine the transformations of P related to minerals in the sediments.

Ca K-edge XANES is a useful tool to study the compositions of Ca in suspended fluvial sediments. $Ca_3(PO_4)_2$, Ca_Pectate, and Calcite are selected as references for LCF, as they are common in soils and sediments (Figure 4.9). $Ca_3(PO_4)_2$ and calcite are inorganic Ca compounds, while $Ca_3(PO_4)_2$ represents Ca-P. Ca_Pectate is an organic Ca compound. The reference spectra show that calcite has a double-peak at the main edge and a shoulder peak around 4060 eV, which are different from the other references. $Ca_3(PO_4)_2$ also has a double-peak feature at the edge jump around 4045 eV, similar to that of calcite. Both peaks of pectate and $Ca_3(PO_4)_2$ appear at 4050 eV, while Ca_Pectate has one main edge peak without the edge jump at 4045 eV.

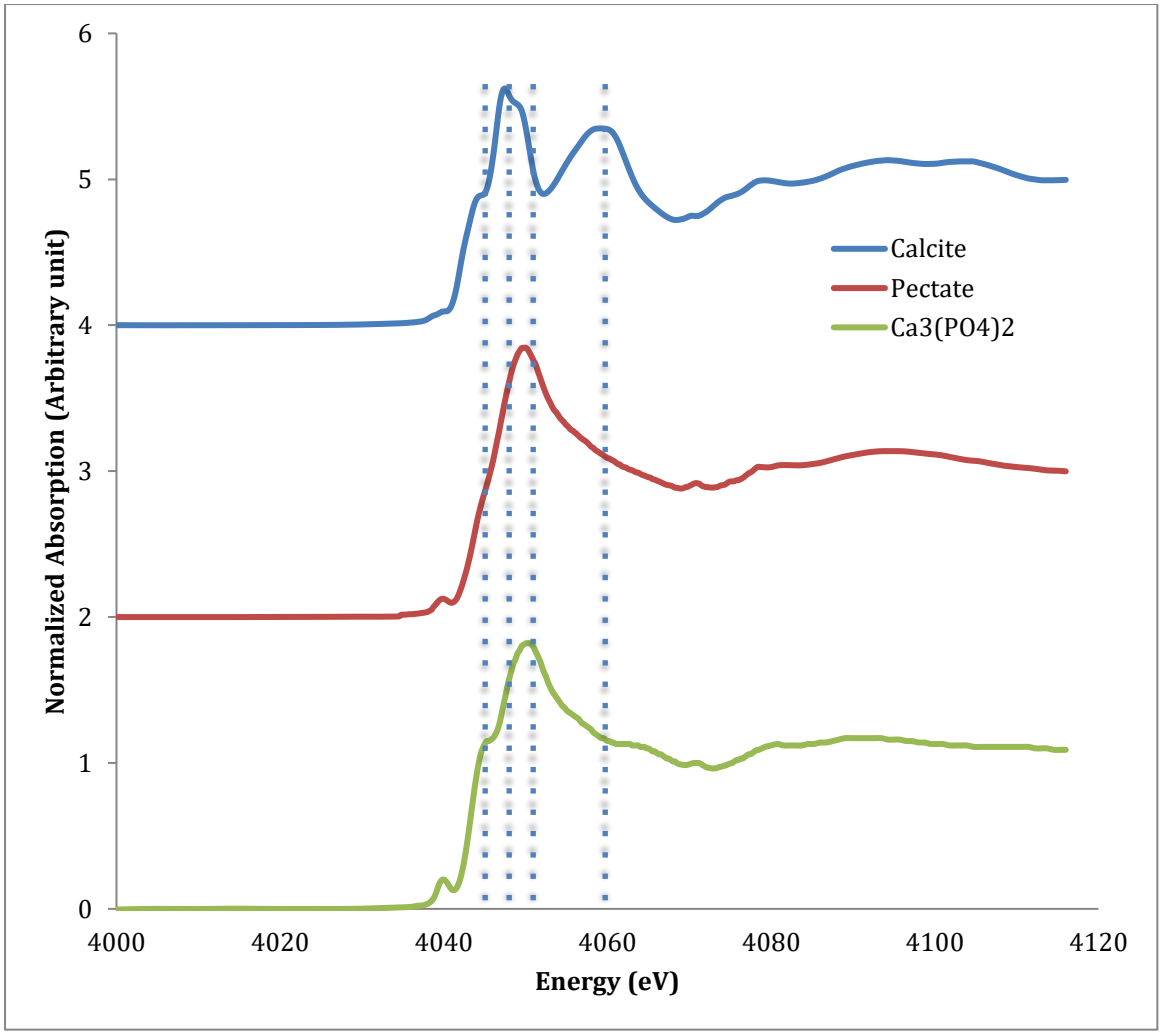

Figure 4.9 Normalized Ca K-edge XANES spectra for reference Ca compounds

Figure 4.10 shows the Ca-K edge XANES spectra for all the samples. It is easy to distinguish that both fluvial sediments from BB streams have two peaks at main edge area, which indicates the presence of Calcite. This observation agrees with the geological information that the soils from BB catchment contain limestone. The other spectra are quite similar to the organic Ca reference spectrum, which indicates that organic Ca dominates those samples. The "disappearance" of calcite after the DCDA experiment, particularly at the BB site, suggests the loss of calcite during the DCDA experiment, in agreement with the XRF results (Figure 4.4). The fittings have small R-factor values (<<0.05) (Table 4.4). The detailed LCF compositions results are shown in Figure 4.11.

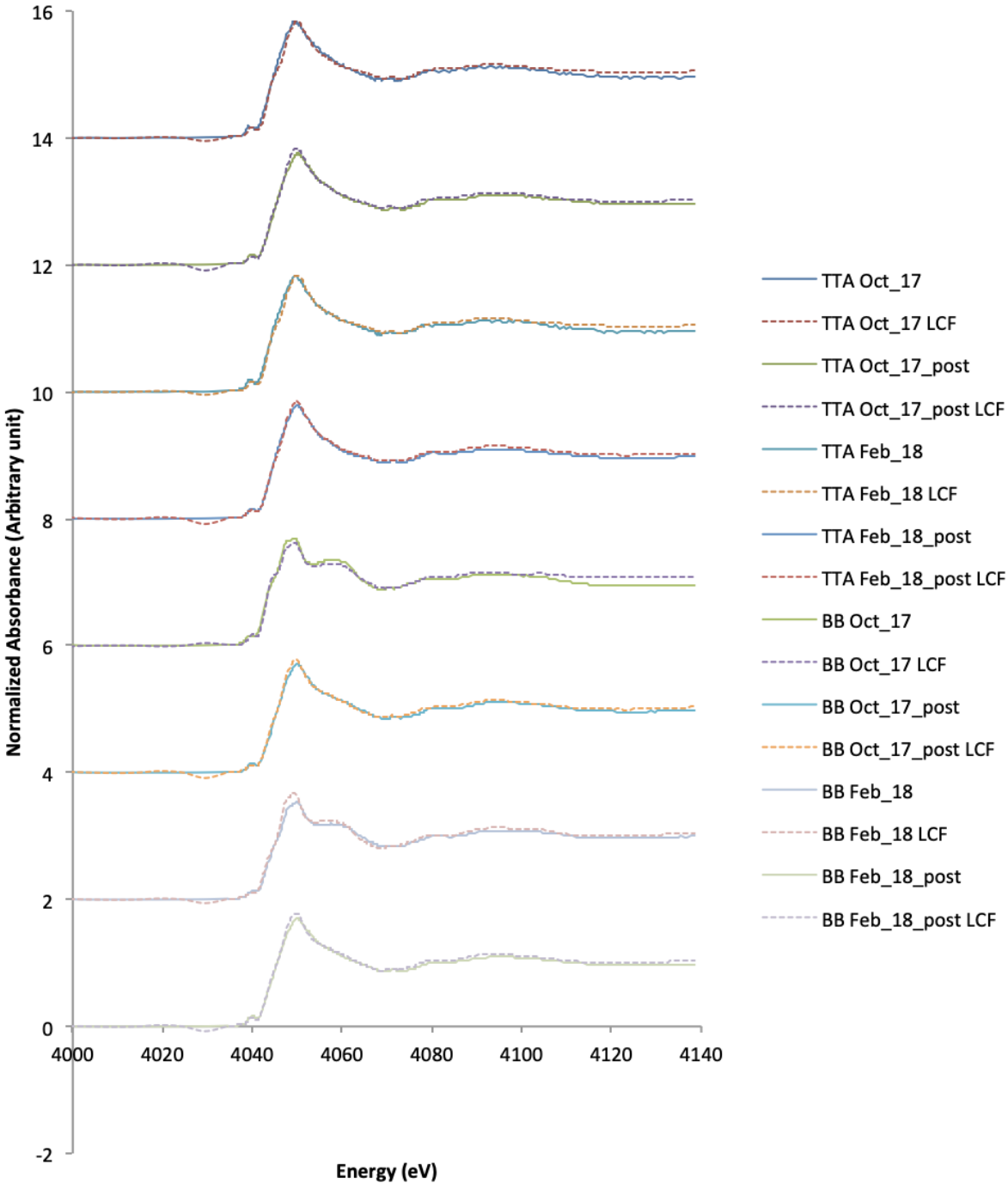

Figure 4.10 Normalized Ca K-edge XANES spectra and LCF spectra for the fluvial sediments of TTA and BB catchments streams pre- and post DCDA experiments, the spectra were offset for a better comparison

Table 4.4 R-factor values for each Ca K-edge XANES LCF fitting to the original spectrum

Sample	TTA Oct_17	TTA Oct_17_post	TTA Feb_18	TTA Feb_18_post	BB Oct_17	BB Oct_17_post	BB Feb_18	BB Feb_18_post
R-factor	0.0171	0.0045	0.0162	0.0031	0.0123	0.0043	0.0159	0.0048

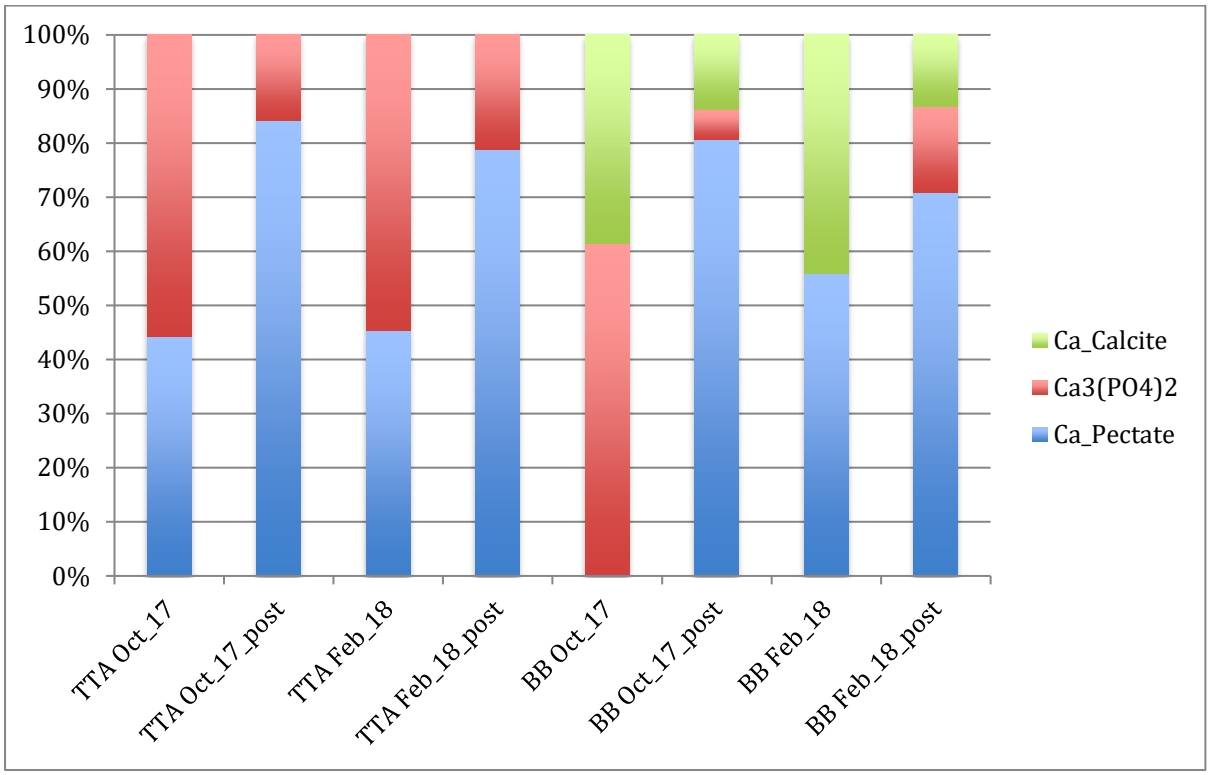

Figure 4.11 LCF Composition results for Ca in samples

LCF compositions show that BB pre-DCDA samples contain calcite, which agrees with the geological information. Since Ca_Calcite fraction decreased after DCDA experiments, it indicates that Ca_Calcite is easily biodegradable, perhaps also soluble during the DCDA. Even though the fraction of $Ca_3(PO_4)_2$ in TTA decreased after DCDA experiments, the presence of $Ca_3(PO_4)_2$ in all post-DCDA samples suggests that $Ca_3(PO_4)_2$ is not completely biodegradable, which agrees with the increased fraction of $Ca_3(PO_4)_2$ in P-K edge XANES results. Ca_Pectate dominates all the post- DCDA samples and pre-DCDA TTA samples indicate that organic Ca is the most persistent Ca-compound in fluvial sediments. The fraction changes of organic Ca compound and inorganic Ca compounds also confirm that the chelation of metal ions with organic matters inhibit biodegradations of sediments.

Fe K-edge XANES is used to study the transformation of Fe speciation of fluvial sediments before and after the DCDA experiment. Several reference spectra of the common

Fe compounds present in agricultural soils and sediments are compared with the sample spectra to identify the compostions of the samples. After comparison with samples, $FePO_4 \bullet 2H_2O$, Ferrihydrite, Geothite, and Fe(III) Oxalate, are selected for LCF. Fe(III) Oxalate is an organic compound while the others are inorganic Fe compounds, and $FePO_4 \bullet 2H_2O$ represents Fe-P. Figure 4.12 shows the selected references spectra. Unlike P-K dege reference spectra, Fe-K edge spectra are rather similar, with small differences in the pre-edge peak at 7115 eV (slightly lower for the organic Fe species (Fe(III) oxalate), and the lack of post-edge bump around 7145 eV for $FePO_4 \bullet 2H_2O$.

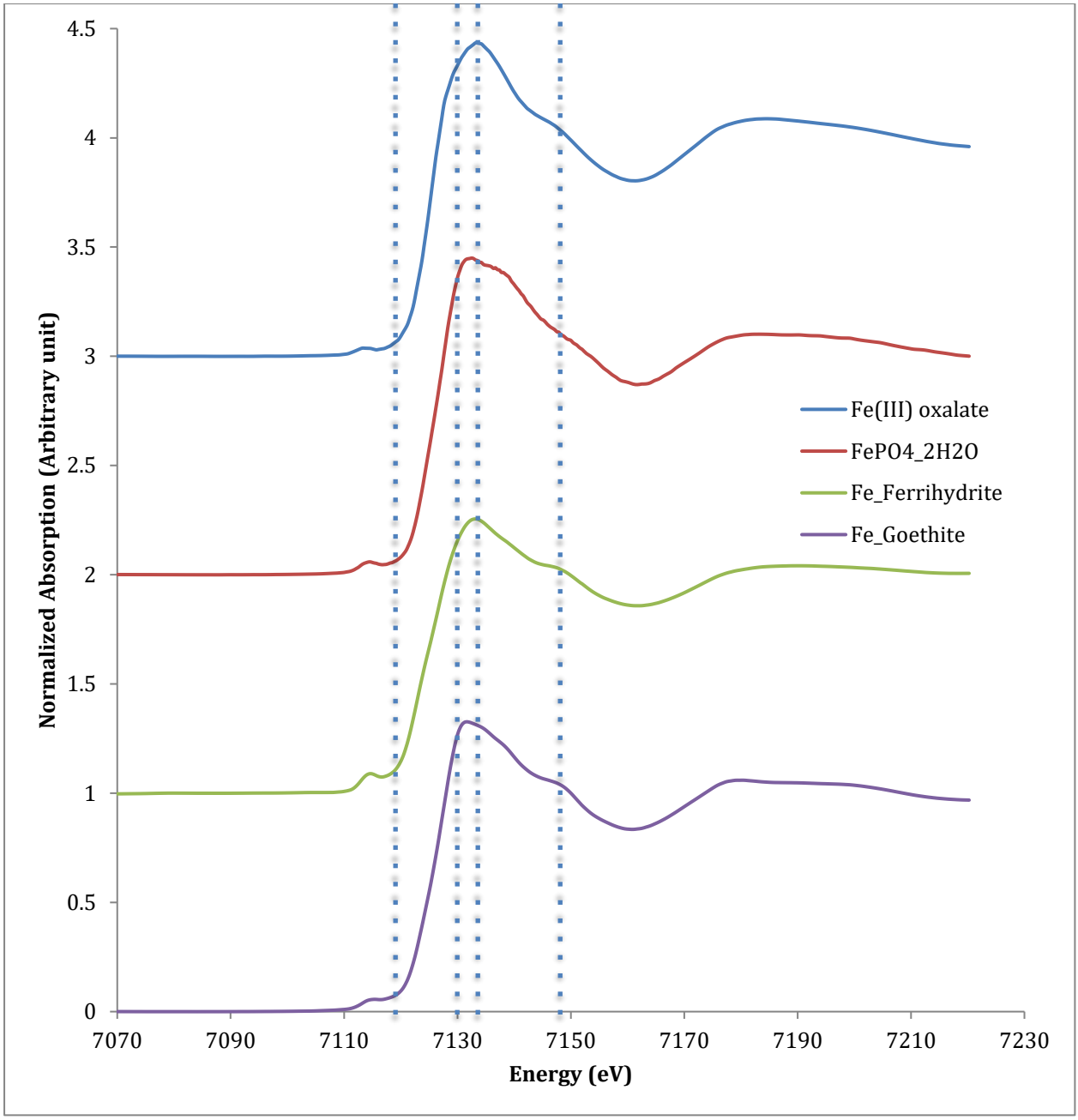

Figure 4.12 Normalized Fe K-edge XANES spectra for Fe reference compounds

Figure 4.13 includes the Fe K-edge spectra for all the pre- and post- DCDA samples. The fluvial sediment samples collected from TTA in October 2017 show different features from the other samples in that the pre-edge peak is higher, and the peak is not as "sharp" as the others. Therefore, it is expected to see that TTA Oct17 sample has quite different Fe compositions from other samples.

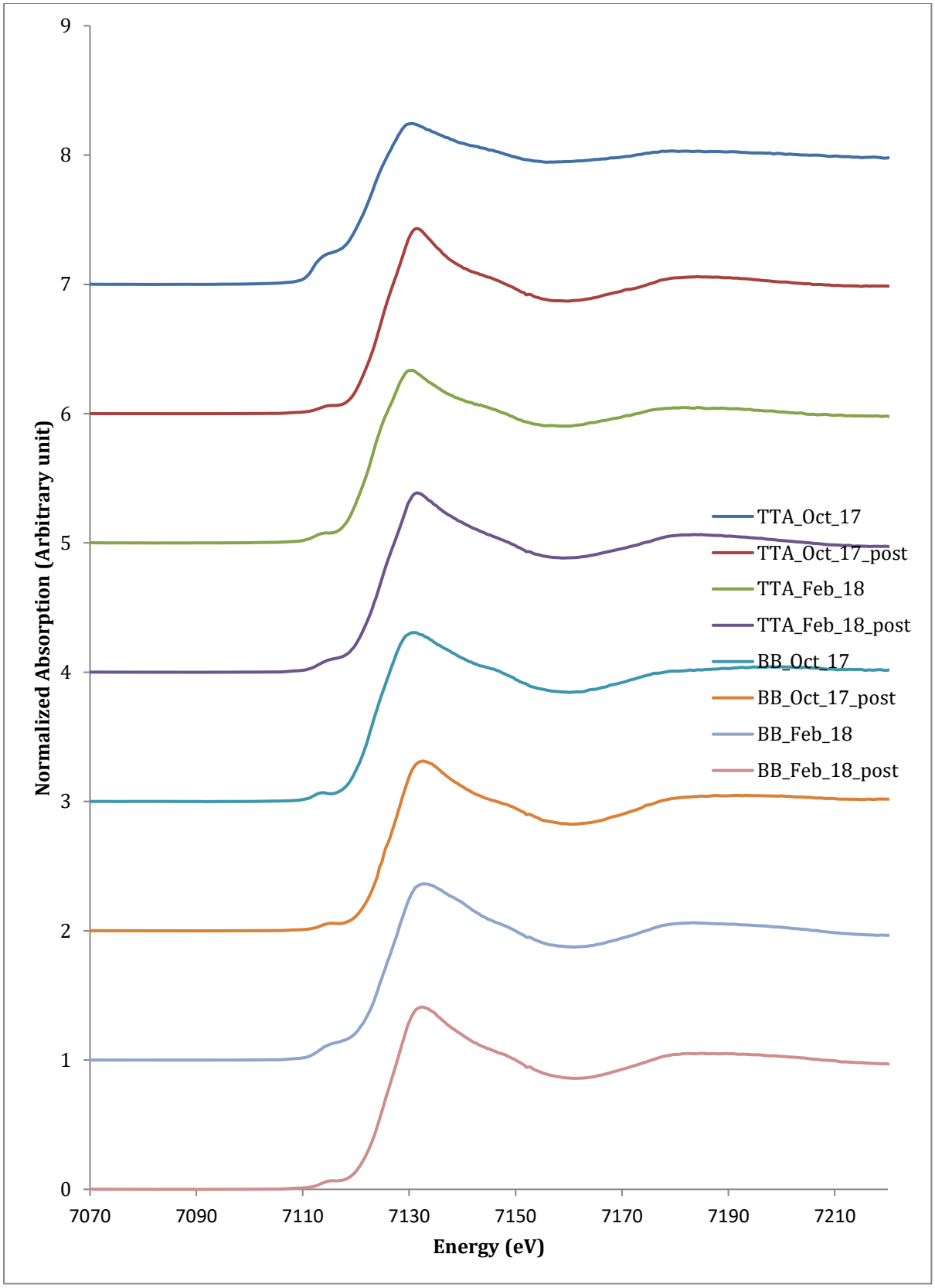

Figure 4.13 Normalized Fe-K Edge XANES spectra for the fluvial sediments samples of TTA and BB catchments streams pre- and post DCDA experiments

Unfortunately, after a few tests of LCF for the Fe compositions in samples, it is unable to generate high quality LCF results for Fe. The poor fittings of Fe compounds indicate that Fe minerals dominate the total Fe fractions in fluvial sediments. Considering the XRF results that all samples have high Fe element, the Fe-P fraction may be too little to be resolved, and the Fe K-edge XANES spectra may not be sensitive to track the change of Fe-P in these experiments.

4.5 Summary of Results and Discussion

Overall, all the above analyses and results show that more bioavailable P is transformed within fluvial suspended sediments during drought events. The geological differences of the BB and TTA catchments affect the biogeochemical transformations of P species and forms within the fluvial suspended sediments from both catchment streams. In general, samples from BB catchment have more TP and bioavailable P compared to samples from TTA catchment. In case of P speciation, all loosely sorbed P, parts of Al-P and OP are easily bioavailable P, while Fe-P is more likely to form and slow rates of organic matter degradation. The differences of Ca-P changes in CSE and the reduction of elemental Ca amounts in XRF between the catchments suggest different transformation mechanisms of the two catchments, as the BB catchment has large amount of calcite, which is also confirmed by Ca K-edge XANES. The discrepancies of Fe-P increases from CSE, the elemental Fe changes from XRF, and the $FePO_4\_2H_2O$ changes from the P K-edge XANES spectrum analysis indicate that Fe-P in fluvial sediments could limit the P biodegradation at some level within the fluvial suspended solids, but all the original fluvial sediment samples are dominated with inorganic Fe complexes, and the total Fe-P concentrations are little compared to the other forms of Fe. By fingerprinting, it is found that phytic acid is the main contributor to bioavailable P. However, Ca K-edge XANES analyses show that inorganic Ca compounds are easily transformed in comparison with organic Ca compounds, which confirm that Ca

bonding to organic matters inhibits biodegradations of organic matter within the suspended sediments. Furthermore, there are some evidences to suggest that $CaCO_3$-P is more likely to be affected by the change in pH, which highlights the importance of monitoring climate change, as it could hugely affect the downstream eutrophication in a negative way.

The results confirm the feasibility of the conjunctive study in bioavailable P of suspended sediments. Not only the values could be applied to future source tracing and mapping, but also the methods could be used to study more suspended sediments from agricultural catchments worldwide. Additionally, the findings, such as the reduction of biodegradation due to the interactions of metal oxides with organic P and the sensitivity of $CaCO_3$-P to the pH changes, could be used to help address the eutrophication treatment and monitoring in the future.

6 Conclusion and Recommendation

6.1 Conclusion

This thesis shows a conjunctive study of a Dual Culture Diffusion Apparatus (DCDA) experiment, chemical sequential extractions (CSE), X-Ray Fluorescence (XRF) Spectroscopy and X-ray Absorption Near-edge Structure (XANES) Spectroscopy, to understand internal P transformations in suspended fluvial sediments. These analytic methods complement each other, and provide an in-depth understanding of P speciation mechanisms.

In general, larger catchment area can be related to higher amounts of total phosphorus (TP) load, and more bioavailable P being "stored" in sediments during "drought" conditions. In terms of P fractions, the loose-P, Al-P and OP are the main contributors to bioavailable P, whereas Ca-P and Fe-P limit the P release at some levels. Notably, the mechanisms for P transformation of Ca-P and Fe-P are quite different. Based on the conjunctive analyses of the CSE, XRF and XANES, it is clear that Fe-P, as $FePO_4\_2H_2O$ in the sediments may prevent organic matter within the suspended sediments from biodegradations. While mineral Fe dominates the total Fe in sediments, the Fe-P amounts are quite small in all of the samples compared to the total Fe amounts. In terms of Ca-P, the different Ca-P trends between the two catchments indicate that pH and calcite plays different roles in P transformation mechanisms than other Ca-P compounds, i.e. organic Ca most likely inhibits biodegradation of organic matter within the suspended sediments, while $Ca_3(PO_4)_2$ can contribute to bioavailable P. The amounts of bio-transferable P in sediments are affected by different aspects, such as the pH and the flow rate of the rivers. Overall, the chelation of metal ions oxides with organic matters is one of the major inhibitors of biodegradations of sediments.

This study provides a comprehensive method to study the internal phosphorus transformation for different catchments, with correlations of local weather and geological

information. The results will benefit future source tracing and modeling, and thus contribute to land management.

6.2 Recommendation

While our understanding of P speciation in fluvial sediments from agricultural catchment has been improved through the conjunctive applications of DCDA, CSE, XRF, and XANES, more detailed information on organic P is still needed to be discovered.

To complete the study, future studies could use ^{31}P Nuclear Magnetic Resonance (NMR) spectroscopy technique as a tool to investigate the organic P compounds in fluvial sediments. Thus, more detailed P speciation would be generated, and will benefit source tracing in terms of expanding sources characterizations.

More information of the farm activities, such as fertilizer application and cattle types, should be investigated according to the sampling times. This information could expand our understanding in P sources, and the mechanism of P transformation during DCDA experiments.

More samples during drought/storm events should be collected and analyzed, as one sediment sample might not be representative for the whole season.

References

Acksel, A., Baumann, K., Hu, Y., & Leinweber, P. (2019). A critical review and evaluation of some P-research methods. *Communications in Soil Science and Plant Analysis , 50* (22), 2804-2824.

Ahlgren, J., Tranvik, L., Gogoll, A., Waldeback, M., Markides, K., & Rydin, E. (2005). Sediment depth attenuation of biogenic phosphorus compounds measured by 31P NMR. *Environmental Science & Technology , 39*, 867-872.

Ajiboye, B. (2007). *Molecular Speciation of Phosphorus in Organic Amendments and Amended Soils Using Nuclear Magnetic Resonance and X-Ray Absorption Spectroscopies.* Manitoba: University of Manitoba.

Apel, P. (2001). Track etching technique in membrane technology. *Radiation Measurement , 34* (1-6), 559-566.

Cade-Menun, B. J. (2006). Characterizing phosphorus in environmental and agricultural samples by 31P nuclear magnetic resonance spectroscopy. *Talanta , 66* (2005), 359-371.

Deacon, J. (2015). *Determining biologically available phosphorus in storm water entering Lake Whatcom, WA using the dual culture diffusion apparatus.* Retrieved from WWU Graduate School Collection: https://cedar.wwu.edu/wwuet/397

Defforey, D., & Paytan, A. (2018). Phosphorus cycling in marine sediments: Advances and challenges. *Chemical Geology , 477*, 1-11.

DePinto, J. V., Young, T. C., & Martin, S. C. (1981). Algal-available Phosphorus in Suspended Sediments from Lower Great Lakes Tributaries. *Journal of Great Lakes Research , 7*, 311-325.

Ellison, M. E., & Brett, M. T. (2006). Particulate phosphorus bioavailability as a function of stream flow and land cover. *Water Research , 40* (6), 1268-1268.

Environment Protection Agency of Ireland. (n.d.). *EPA Maps*. Retrieved January 8, 2020, from Environment Protection Agency: https://gis.epa.ie/EPAMaps/

Environment Protection Agency of Ireland. (2018). *Water Quality in 2017: An Indicators Report.* Dublin: Environment Protection Agency of Ireland.

Eusterhues, K., Neidhardt, J., Hädrich, A., Küsel, K., & Totsche, K. U. (2014). Biodegradation of ferrihydrite-associated organic matter. *Biogeochemistry , 119* (1-3), 45-50.

Ghosh, P., Rathinasabapathi, B., & Ma, L. Q. (2015). Phosphorus solubilization and plant growth enhancement by arsenic-resistant bacteria. *Chemosphere , 134*, 1-6.

Graf, E. (1983). Applications of phytic acid. *Journal of the American Oil Chemists' Society , 60*, 1861-1867.

Great Lakes WATER Institute. (2004). *Cladophora Research and Management in the Great Lakes.* University of Wisconsin-Milwaukee, Department of BIological Science. Milwaukee: University of Wisconsin-Milwaukee.

Gu, C., Hart, S. C., Turner, B. L., Hu, Y., Meng, Y., & Zhu, M. (2019). Aeolian dust deposition and the perturbation of phosphorus transformations during long-term ecosystem development in a cool, semi-arid environment. *Geochimica et Cosmochimica Acta , 246*, 498-514.

Hansel, C. M., Benner, S. G., & Fendorf, S. (2005). Competing Fe(II)-induced mineralization pathways of ferrihydrite. *Environmental Science & Technology , 39*, 7147-7153.

Hoffman, R. E. (2003). Variations on the chemical shift of TMS. *Journal of Magnetic Resonance , 163* (2), 325-331.

Huang, C. (2015). *Understanding Chemical Sequential Extraction Method by Using Nuclear Magnetic Resonance and X-Ray Absorption Near Edge Spectroscopies for*

Phosphorus Fractionation of Lake Sediments. MSc Thesis, University of Saskatchewan, Civil and Geological Engineering, Saskatoon.

Hupfer, M., Zak, D., Rossberg, R., Herzog, C., & Pothig, R. (2009). Evaluation of a well-established sequential phosphorus fractionation technique for use in calcite-rich lake sediments: identification and prevention of artifacts due to apatite formation. *Limnology and Oceanography-Methods , 7*, 399-410.

Jahrman, E. (2020). *Xray Absorption Near Edge Spectroscopy-XANES*. Retrieved February 7, 2020, from Clean Energy Institute: https://www.cei.washington.edu/education/science-of-solar/xray-absorption-near-edge-spectroscopy-xanes/

Jennings, E., Mills, P., Jordan, P., Jensen, J.-P., Søndergaard, M., Barr, A., et al. (2003). *Eutrophication from Agricultural Sources: Seasonal Patterns & Effects of Phosphorus.* Environmental Protection Agency. Wexford, Ireland: An Ghníomhaireacht um Chaomhnú Comhshaoil.

Kim, D.-G., Park, J., Lee, D., & Kang, H. (2011). Removal of Nitrogen and Phosphorus from Effluent of a Secondary Wastewater Treatment Plant Using a Pond-Marsh Wetland System. *Water, Air, & Soil Pollution , 214*, 37-47.

Kraal, P., Kijkstra, N., Behrends, T., & Slomp, C. P. (2017). Phosphorus burial in sediments of the sulfidic deep Black Sea: Key roles for adsorption by calcium carbonate and apatite authigenesis. *Geochimica et Cosmochimica Acta , 204*, 140-158.

Kruse, J., Abraham, M., Amelung, W., Baum, C., Bol, R., Kuhn, O., et al. (2015). Innovative methods in soil phosphorus research: A review. *J. Plant Nutr. Soil Sci. , 178*, 43-88.

Li, G., Li, H., Leffelaar, P., Shen, J., & Zhang, F. (2014). Characterization of phosphorus in animal manures collected from three (dairy, swine, and broiler) farms in China. *Plus One , 9* (7).

Liu, J., Yang, J., Cade-Menun, B. J., Hu, Y., Li, J., Peng, C., et al. (2017). Molecular speciation and transformation of soil legacy phosphorus with and without longterm phosphorus fertilization: Insights from bulk and microprobe spectroscopy. *Scientific Reports , 7* (15354).

Lukkari, K., Leivuori, M., & Hartikainen, H. (2007). Fractionation of sediment phosphorus revisited: II Changes in phosphorus fractions during sampling and storing in the presence or absence of oxygen. *Limnology and Oceanography: Methods , 5*, 445-456.

Mayer, T. (1985). *A review of procedures for evaluation of bioavailable phosphorus in particulate materials.* National Water Research Institute, Aquatic Ecology Division. Burlington: National Water Research Institute.

Mayer, T. (1991). *Rapid procedures for determining bioavailable and total phosphorus in freshwater sediments.* Canadian National Water Research Institute, River Research Branch. Burlington: Canadian National Water Research Institute.

Miller, W. E., Green, J. C., & Shiroyama, T. (1978). *The Selenastrum Capricornutum Printz Algal Assay Bottle Test, Experimental Design, Application, and Data Interpretation Protocal.* Corvallis, Oregon: US EPA.

Miller, W. E., Greene, J. C., & Shiroyama, T. (1978). *The Selenastrum Capricornutum Printz Algal Assay Bottle Test Experimental Design, Application, and Data Interpretation Protocol.* Oregon, USA: U.S. Environmental Protection Agency.

Nelson, J. H. (2003). *Nuclear magnetic resonance spectroscopy.* Upper Saddle River, NJ: Prentice Hall.

Nover. (2004). Management Application and Measurement of Bioavailable Particulate Phosphorus. 17.

O'Connell, D. W., Ansems, N., Kukkadapu, R. K., Jaisi, D., Orihel, D. M., Cade-Menum, B. J., et al. (2020). Sedimentary phosphorus speciation dynamics following artificial

eutrophication of Lake 227, Experimental Lakes Area, Ontario, Canada. *Journal of Geophysical Research: Biogeosciences* .

Orihel, D. M., Baulch, H. M., Casson, N. J., North, R. L., Parsons, C. T., Seckar, D. C., et al. (2017). Internal phosphorus loading in Canadian fresh waters: A critical review and data analysis. *Canadian Journal of Fisheries and Aquatic Sciences , 74*, 2005-2029.

Pettersson, K., Bostrom, B., & Jacobsen, O.-S. (1988). Phosphorus in sediments — speciation and analysis. *Hydrobiologia , 170*, 91-101.

Psenner, R., Pucsko, R., Sager, M., & Univ. Innsbruck, A. (1984). The fractionation of organic and inorganic phosphorus coumpounds in sediments; an attempt to characterize ecologically inportant fractions. *Archiv Fuer Hydrobiologies , 70* (1), 111-155.

Ravel, B., & Newville, M. (2005). ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. *Journal of Synchrotron Radiation , 12*, 537-541.

Residue. (n.d.). *Cambridge Dictionary*. Retrieved June 29, 2020, from https://dictionary.cambridge.org/dictionary/learner-english/residue

Rydin, E. (2000). Potentially mobile phosphorus in Lake Erken sediment. *Water Research , 34* (7), 2037-2042.

Soliman, N. F., El Zokm, G. M., & Okbah, M. A. (2017). Evaluation of phosphorus bioavailability in El Mex Bay and Lake Mariut sediments. *International Journal of Sediment Research , 32* (3), 432-441.

Sondergaard, M., Jensen, J. P., Jeppesen, E., & Hald Moller, P. (2002). Seasonal dynamics in the concentrations and retention of phosphorus in shallow Danish lakes after reduced loading. *Aquatic Ecosystem Health & Management , 5*, 29-39.

The Irish Meteorological Service. (N/A). *30 YEAR AVERAGES.* Retrieved June 30, 2020, from The Irish Meteorological Service: https://www.met.ie/climate/30-year-averages#

Ulrich, A. E., Malley, D. F., & Watts, P. D. (2016). Lake Winnipeg Basin: Advocacy, challenges and progress for sustainable phosphorus and eutrophication control. *Science of The Total Environment , 542*, 1030-1039.

United States Environmental Protection Agency. (1971). *Algal Assay Procedure Bottle Test.* Corvallis: United States Environmental Protection Agency.

Usask. (2014, May 6). *How a Synchrotron Works*. Retrieved September 24, 2019, from Youtube: https://www.youtube.com/watch?v=nuJnJqq9LXM

Verma, H. R. (2007). X-Ray Fluorescence (XRF) and Particle-Induced X-ray Emission (PIXE). In H. R. Verma, *Atomic and Nuclear Analytical Methods: XRF, Mossbauer, XPS, NAA and B63Ion-Beam Spectroscopic Techniques* (pp. 1-90). Berlin: Springer.

Wang, H.-Z., & Wang, H.-J. (2019). Eutrophication: a limiting nutrient is not necessarily an abating factor. *Science Bulletin , 64* (16), 1125-1128.

Wang, T., Camps-Arbestain, M., Hedley, M., & Bishop, P. (2012). Predicting phosphorus bioavailability from high-ash biochars. *Plant and Soil , 357*, 173-187.

Werner, F., & Prietzel, J. (2015). Standard Protocol and Quality Assessment of Soil Phosphorus Speciation by P K-Edge XANES Spectroscopy. *Environmental Science & Technology , 49* (17), 10521-10528.

Wilson, M. A. (1987). *N.M.R. Techniques and applications in geochemistry and soil chemistry.* Oxford, UK: Pergamon Press.

World Resources Institute. (2020). *Sources of Eutrophication*. Retrieved April 17, 2020, from World Resources Institute: https://www.wri.org/our-work/project/eutrophication-and-hypoxia/sources-eutrophication

Worsfold, P. J., & et al. (2005). Sampling, sample treatment and quality assurance issues for the determination of phosphorus species in natural waters and soils. *Talanta , 66*, 273-293.

Yamagishi, T., Yamaguchi, H., Suzuki, S., Horie, Y., & Tatarazako, N. (2017). Cell reproductive patterns in the green alga Pseudokirchneriella subcapitata (=Selenastrum capricornutum) and their variations under exposure to the typical toxicants potassium dichromate and 3,5-DCP. *PLoS ONE , 12* (2).

Yu, Y., Song, J., Li, X., Yuan, H., & Li, N. (2012). Distribution, sources and budgets of particulate phosphorus and nitrogen in the East China Sea. *Continental Shelf Research , 43*, 142-155.

Zhang, H., Shi, L., Lu, H., Shao, Y., Liu, S., & Fu, S. (2020). Drought promotes soil phosphorus transformation and reduces phosphorus bioavailability in a temperate forest. *Science of The Total Environment , 732*.

Appendix

Table A.1 Summary of P-free Algae Growth Medium Nutrients (United States Environmental Protection Agency, 1971)

Nutrients		**Concentration (g/L)**
Sodium Nitrate	$NaNO_3$	25.5
Sodium Bicarbonate	$NaHCO_3$	15
Magnesium Sulphate	$MgSO_4 \bullet 7H_2O$	14.7
Magnesium Chloride	$MgCl_2 \bullet 6H_2O$	12.2
Calcium Chloride	$CaCl_2 \bullet 2H_2O$	4.41
Potassium Chloride	KCl	0.894
Trace Metal+EDTA		Combined micronutrients (Table B)
1 mL of each nutrient in 1 L of milli-Q water		

Table A.2 Summary of Trace Metal+EDTA solution (United States Environmental Protection Agency, 1971)

Trace Metal	**Concentration (mg/L)**
H_3BO_3	186
$MnCl_2 * 4H_2O$	414
$ZnCl_2$	3.27
$CoCl_2 * 6H_2O$	1.43
$CuCl_2 * 2H_2O$	0.011
$Na_2MoO_4 * 2H_2O$	7.26
$FeCl_3$	96.0
$NaEDTA \bullet 2H_2O$	300

Table A.3 Summary of Chemical Sequential Extractions Results (mg/g)

	TTA Oct_17	TTA Oct_17_post	TTA Feb_18	TTA Feb_18_post	BB Oct_17	BB Oct_17_post	BB Feb_18	BB Feb_18_post
1st Loose-P	0	0	0.02	0	0.31	0.01	0.35	0
2nd Loose-P	0.02	0	0	0	0.41	0.01	0.45	0.05
1st Fe/Mn-P	0.03	0.2	0.02	0.14	0.25	0.29	0.11	0.52
2nd Fe/Mn-P	0.05	0.27	0.02	0.19	0.33	0.39	0.17	0.57
1st Al-P	0.41	0.4	0.39	0.28	1.03	0.34	0.4	0.02
2nd Al-P	0.49	0.35	0.33	0.3	0.93	0.38	0.44	0.02
1st OP	0.3	0.2	0.12	0.04	0.42	0.2	0.29	0.1
2nd OP	0.47	0.3	0.18	0.08	0.46	0.34	0.37	0.14
1st Ca-P	0.2	0.05	0.1	0.03	0.01	0.09	0.04	0.05
2nd Ca-P	0.1	0.08	0.12	0.05	0.01	0.12	0.07	0.08
1st Other-P	0.36	0.24	0.21	0.18	0.41	0.11	0.69	0.11
2nd Other-P	0.35	0.27	0.22	0.13	0.49	0.13	0.81	0.29

Copyright Permissions for reprinted Figures and Tables

SPRINGER NATURE LICENSE
TERMS AND CONDITIONS
Sep 27, 2020

This Agreement between Ms. Qingxin Zhang ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4914120156720
License date	Sep 22, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Springer eBook
Licensed Content Title	X-ray Fluorescence (XRF) and Particle-Induced X-ray Emission (PIXE)
Licensed Content Date	Jan 1, 2007
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	Speciation of Bioavailable Phosphorus in Fluvial Sediments
Institution name	University of Saskatchewan
Expected presentation date	Sep 2020
Portions	Figure 1.4
Requestor Location	Ms. Qingxin Zhang 2710 Main Street Suite 232 Saskatoon, SK S7H 0M3 Canada Attn: Ms. Qingxin Zhang
Total	0.00 CAD

ELSEVIER LICENSE
TERMS AND CONDITIONS

Sep 27, 2020

This Agreement between Ms. Qingxin Zhang ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	4913381314071
License date	Sep 20, 2020
Licensed Content Publisher	Elsevier
Licensed Content Publication	Talanta
Licensed Content Title	Characterizing phosphorus in environmental and agricultural samples by 31P nuclear magnetic resonance spectroscopy
Licensed Content Author	Barbara J. Cade-Menun
Licensed Content Date	Apr 15, 2005
Licensed Content Volume	66
Licensed Content Issue	2
Licensed Content Pages	13
Start Page	359
End Page	371
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	2
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title	Speciation of Bioavailable Phosphorus in Fluvial Sediments
Institution name	University of Saskatchewan
Expected presentation date	Sep 2020
Portions	Figure 1, Table 1
Requestor Location	Ms. Qingxin Zhang 2710 Main Street Suite 232 Saskatoon, SK S7H 0M3 Canada Attn: Ms. Qingxin Zhang
Publisher Tax ID	GB 494 6272 12
Total	0.00 USD

ELSEVIER LICENSE
TERMS AND CONDITIONS

Sep 27, 2020

This Agreement between Ms. Qingxin Zhang ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	4913370822301
License date	Sep 20, 2020
Licensed Content Publisher	Elsevier
Licensed Content Publication	Journal of Great Lakes Research
Licensed Content Title	Algal-Available Phosphorus in Suspended Sediments from Lower Great Lakes Tributaries
Licensed Content Author	Joseph V. DePinto,Thomas C. Young,Scott C. Martin
Licensed Content Date	Jan 1, 1981
Licensed Content Volume	7
Licensed Content Issue	3
Licensed Content Pages	15
Start Page	311
End Page	325
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title	Speciation of Bioavailable Phosphorus in Fluvial Sediments
Institution name	University of Saskatchewan
Expected presentation date	Sep 2020
Order reference number	1
Portions	Figure 2
Requestor Location	Ms. Qingxin Zhang 2710 Main Street Suite 232 Saskatoon, SK S7H 0M3 Canada Attn: Ms. Qingxin Zhang
Publisher Tax ID	GB 494 6272 12
Total	0.00 CAD

From: **Peng, Jian** jip601@mail.usask.ca
Subject: Re: Request for Copyright Permission for Reuse in a Thesis/Dissertation
Date: September 29, 2020 at 10:09 AM
To: Wegner, Leon leon.wegner@usask.ca, Zhang, Qingxin qiz393@mail.usask.ca
Cc: Hanke, Cynthia cynthia.hanke@usask.ca

JP

Dear Qingxin Zhang:
You are welcome to reuse the figures.
Sincerely,
Jian Peng

From: Wegner, Leon <leon.wegner@usask.ca>
Sent: September 28, 2020 10:06 AM
To: Zhang, Qingxin <qiz393@mail.usask.ca>
Cc: Hanke, Cynthia <cynthia.hanke@usask.ca>; Peng, Jian <jip601@mail.usask.ca>
Subject: RE: Request for Copyright Permission for Reuse in a Thesis/Dissertation

Dear Qingxin Zhang:

As permission must first be sought from the supervising professor, I am forwarding your request to Professor Jian Peng, who supervised the thesis work that you are seeking permission to copy.

Professor Peng, please respond directly to Qingxin to provide permission.

Sincerely,

Leon D. Wegner, Ph.D., P.Eng.
Professor and Head
Department of Civil, Geological, and Environmental Engineering
College of Engineering, University of Saskatchewan
Room 3B48.3, Engineering Building, 57 Campus Drive
Saskatoon, SK S7N 5A9
CANADA
Tel: (306)**966-5349** Fax: (306) **966-5205**

From: Zhang, Qingxin <qiz393@mail.usask.ca>
Sent: Sunday, September 27, 2020 11:35 AM
To: Wegner, Leon <leon.wegner@usask.ca>
Subject: Request for Copyright Permission for Reuse in a Thesis/Dissertation

Hi, Head of Department of Civil, Geological, and Environmental Engineering,
I am a M.Sc student of the Department of Chemical & Biological Engineering at the University of Saskatchewan, and am preparing a thesis or dissertation for submission as part of the requirements for my degree at the University of Saskatchewan. I would like to request permissions for reuse figures of a thesis submitted to Department of Civil, Geological, and Environmental Engineering:
Title: Understanding Chemical Sequential Extraction Method by Using Nuclear Magnetic Resonance and X-Ray Absorption Near Edge Spectroscopies for Phosphorus Fractionation of Lake Sediments
Author: Chao Huang

October, 2015

University of Saskatchewan

URI: http://hdl.handle.net/10388/ETD-2015-10-2342

Figure 2.2, Figure 2.4 & Figure 2.5 on page 17-18
The materials may be provided in both print or electronic format. The materials are for reuse in my thesis/dissertation "Speciation of Bioavailable Phosphorus in Fluvial Sediments", which is expected to be presented on October 2020. My thesis will be publicly available in University of Saskatchewan's online digital repository, and I will be granting non-exclusive licences to University of Saskatchewan and to Library and Archives Canada.
Thank you for your time and consideration. If you have any questions, or require additional information, please contact me at at qiz393@usask.ca.
Sincerely,
Qingxin Zhang